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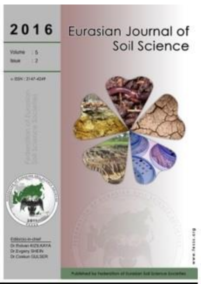

Dissolution of rock phosphate in animal manure soil amendment and lettuce growth

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Abstract

A study was conducted in pots on the field to assess the effect of different quantities of poultry manure (PM), cattle manure (CM) and pig manure (PG) on the release of available phosphorus from Togo rock phosphate (RP) and lettuce growth. There were eleven (11) treatments which were: Control (soil only); 2.5g RP; 2.5g CM; 2.5gRP + 2.5g CM; 2.5gRP + 5gCM; 2.5gPM; 2.5gRP + 2.5gPM; 2.5gRP + 5gPM; 2.5gPG; 2.5gRP + 2.5gPG; 2.5gRP + 5gPG, applied per kg soil, using the Completely Randomized Design (CRD) with three replications. Available phosphorus and other parameters were assessed using standard methods. Results were statistically analyzed using the the GenStat (11th Edition) statistical software package. The amount and type of animal manure in the amendment affected the amount of the available P released. The addition of 2.5g manure to 2.5g RP in a kg of soil significantly ($P<0.05$) increased available P by 4 to 7 times over the sole 2.5g RP/kg soil treatment. Doubling the amount of manure in the amendment (5g manure + 2.5g RP) almost doubled the amount of P released, with the poultry manure combinations being more significant. The amount of available P in the soil positively related to the plant height (R^2=63), leaf area (R^2=0.55), dry weight (R^2=0.73) and the percentage P in the leaf (R^2=0.88) of lettuce. The PM at 2.5gRP + 5gPM recorded the highest significant ($P<0.05$) values. The study has provided further basis for manure selection and quantities to be used in enhancing the release of P from rock phosphate. However, investigations need to be continued using nuclear techniques.

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Keywords: Available phosphorus, manure, rock phosphate, soil amendment

Introduction

In comparison with other parts of the world, most soils of Africa are considered as inherently poor. Nutrient mining combined with the inherently low soil fertility has been blamed for the low crop yields in Africa (Shepherd et al. 1996).

It is generally viewed that substantial increases in inorganic fertilizer use are necessary to restore and maintain the fertility of African soils and enhance their productivity (Minot and Benson, 2009). Though inorganic fertilizers have dramatically increased food production worldwide, high costs, poor distribution systems and lack of manufacturing capacity are some of the factors preventing farmers from accessing the fertilizers they need to maintain the health of their farmland (Eurekalert, 2006).

With the rise in costs of industrial fertilizers of which phosphorus fertilizers are integral part in developing countries, it has become necessary to look for alternative phosphorus sources for farming operations.

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Rock phosphate, a naturally occurring mineral source of phosphate, could serve as an alternative source of phosphorus in developing countries. It has been found to be much less expensive than soluble phosphorus fertilizers (Lorion, 2004). However, rock phosphate material has poor solubility when used as a fertilizer in soils.

Treating rock phosphate with organic materials is found to be a technique for enhancing the solubility and the subsequent availability to plants of phosphorus (Zapata and Roy, 2004).

Several studies have been conducted on amending rock phosphates to enhance their rate of dissolution after application to soils. The incorporation of agricultural wastes like poultry manure and cow dung with rock phosphate significantly improved the release of P and performance of crops (Singh and Amberger, 1990; Akande et.al. 2005; Akande et.al. 2008).

In the quest to delve more into the use of organic manure to ease the release of available phosphorus from rock phosphate, the present study was conducted with the objective of assessing the effect of different kinds and quantities of poultry manure, cattle manure and pig manure on the release of available phosphorus and growth of lettuce using Togo rock phosphate.

Material and Methods

An experiment was carried out at the College of Agriculture Education (latitude 07° 04'N; longitude 01° 24'W), University of Education, Winneba Ghana, from May to July, 2014. The soil of the area is classified by the FAO-UNESCO legend as Chromic Luvisol (Asiamah, 1988).

Soil was sampled from the top layer (0 – 15cm) of the College's experimental field and sieved through a 2mm sieve. The sieved soil was amended with dried and ground poultry manure (PM), cattle manure (CM), pig manure (PG) and rock phosphate (RP), which were also sieved through a 2mm sieve at various proportions in pots (diameter - 10cm; 5kg soil capacity). The physical and chemical properties of the soil, animal manures and the rock phosphate are presented in Tables 1 and 2.

Table 1. Some chemical and physical properties of the soil used for the study

pH (1:1)	OC, %	N, %	Ca	Mg	K	Na	OM, %	P, mg/kg	Exch. A(Al+H)	TEB, me/100g	BS, %	Sand, %	Silt, %	Clay, %
			(me/100g)											
6.33	1.18	0.12	4.63	1.6	0.16	0.09	2.04	28.14	0.10	6.48	98.47	78.28	2.21	19.51
												Loamy Sand		

Table 2. Some chemical properties of rock phosphate and animal manures used for the study

	Ca	Mg	K	P	N
	(%)				
Poultry manure	2.14	0.65	0.90	1.77	4.27
Cattle manure	1.98	0.64	0.26	0.71	2.06
Pig manure	0.26	1.40	0.48	1.57	2.13
Rock phosphate	46.82	0.03	0.02	11.04	0.04

The treatments per kg soil were: Control (soil only); 2.5g RP; 2.5g CM; 2.5gRP + 2.5g CM; 2.5gRP + 5gCM; 2.5gPM; 2.5gRP + 2.5gPM; 2.5gRP + 5gPM; 2.5gPG; 2.5gRP + 2.5gPG; 2.5gRP + 5gPG.

There were three replications for the treatments. The Completely Randomized Design (CRD) was used to assign treatments on the field. The treatments were placed on the field for two weeks under rainfall conditions. Samples of soil from the treatments were taken for chemical analyses before two weeks old lettuce (leaf lettuce) seedlings (4plants/pot) were transplanted on them.

The physical and chemical properties of soil samples, animal manures, rock phosphate and plant samples were assessed using standard methods which involved; organic matter (Walkley and Black, 1934), particle size analysis (Bouyoucos, 1962), total nitrogen and total phosphorus (Anderson and Ingram, 1989), total K (IITA, 1985), available phosphorus (Bray and Kurtz, 1945), exchangeable Ca, Mn, K and Na (IITA, 1985) and Al (McLean, 1982).

At 30 days after transplanting the lettuce plants were harvested. Plant heights were measured with a meter rule and a string. The leaf area of the plants was measured by the grid counting method and the dry matter of the plant assessed by the oven dying method. Values of parameters were subjected to analysis of variance (ANOVA) and the Least Significant Difference Test (P<0.05) for the separation of means using the GenStat (11th Edition) statistical software package.

Results and Discussion

Table 3 indicates available P, exchangeable Ca, Fe, Mn and Al released from the rock phosphate soil amendment with different types of animal manure after two weeks of incubation under natural conditions on the field. At the same RP level of 2.5g/soil the animal manure soil amendments recorded significantly (P<0.05) higher values of available P than the RP + Soil and the control.

Table 3. Available P levels and other nutrients after soil amendment

Treatment (per kg soil)	PO_4, mg/kg	Ca, me/100g	Fe, mg/kg	Mn, mg/kg	Al, me/100g
Control	141.96	5.11	66.04	1.43	0.00
2.5g RP	149.29	5.13	66.98	1.44	0.00
2.5g CM	183.56	5.16	70.00	1.45	0.00
2.5gRP + 2.5g CM	212.94	5.15	69.58	1.45	0.00
2.5gRP + 5gCM	298.62	5.20	69.48	1.47	0.00
2.5gPM	208.05	5.47	68.40	1.44	0.00
2.5gRP + 2.5gPM	256.99	5.40	70.71	1.47	0.00
2.5gRP + 5gPM	389.19	5.61	67.97	1.47	0.00
2.5gPG	200.72	5.34	68.01	1.46	0.00
2.5gRP + 2.5gPG	253.32	5.31	70.50	1.47	0.00
2.5gRP + 5gPG	323.09	5.47	69.00	1.48	0.00
LSD	29.19	0.61	8.30	0.50	NA
CV	12.40	4.11	3.22	10.00	NA

Figure 1 shows the estimated amount of available P released from the RP alone in the various treatments. The amount and type of animal manure in the amendment affected the amount of the available P released. The addition of 2.5g manure to 2.5g RP in a kg of soil significantly (P<0.05) increased available P by 4 to 7 times over the sole 2.5g RP/kg soil treatment. Doubling the amount of manure in the amendment (5g manure + 2.5g RP) almost doubled the amount of P released, especially for the poultry and the cattle manures. Significant differences were seen among the different types of animal manures in the P released, especially, at 5g manure amendment, the poultry manure released the highest amount of P which was almost 1.6 and 1.8 times greater than the amount released under the cattle and pig manures respectively. In a similar study using palm oil mill effluent, the release of available P from RP was found to increase with the application of the effluent and the amount applied (Oviasogie and Uzoekwe, 2011), which is observed in the current study.

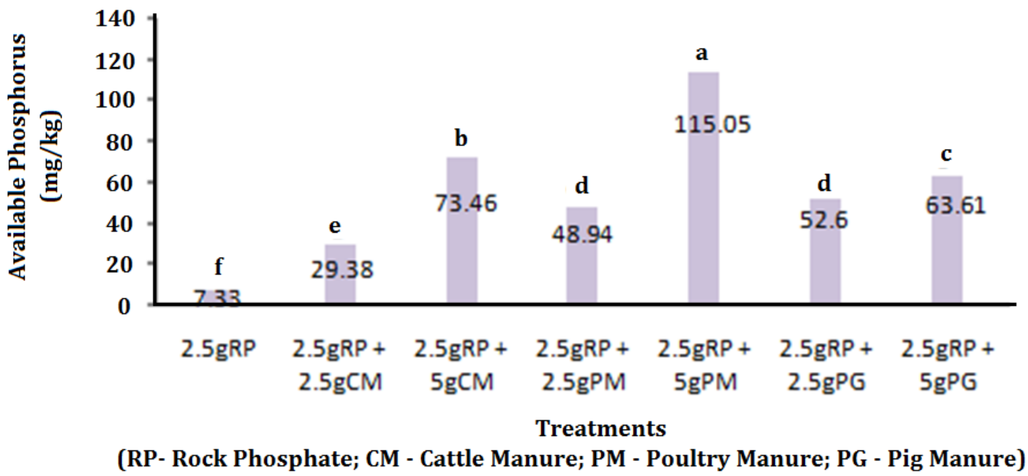

Figure 1. Estimated amount of available phosphorus released from only rock phosphate (RP) under different soil manure amendment (Figures with the same letters are not significantly (P<0.05) different from each other)

The ability of organic manure in enhancing the release of available P from RP has been related to increase in microbial activities and the acidic soil conditions created by the decay of the organic manure (Kim et al. 1997; Rashid et al. 2004; Chen et al. 2006; Nishanth and Biswas, 2008; Kumari et al. 2008). Different organic materials will create different soil environmental conditions (Bangar et al. 1985; Nair and Ngouajio, 2012) and hence leading to differences in the release of available P from RP in soil amendments. Such situations might be the reasons behind the significant performance of the PM in enhancing higher release of available P in the amendments than the rest of the organic materials, because PM has been found to produce higher microbial biomass and hence acidic conditions in soil amendments (Lin et al. 2010).

Available P in the soils may be converted into insoluble complexes (Mehta et al. 2015) through precipitation reactions with Ca^{2+} in calcareous soils and Mn^{3+}, Al^{3+} and Fe^{3+} in acidic soils (Khan et al. 2009). The effect of Ca^{2+} , Mn^{3+}, Al^{3+} and Fe^{3+} impacting differences among the treatments may be considered as insignificant as the concentrations in the treatments were not significantly different (Table 3).

The RP treated with different combinations of the organic manures significantly ($P < 0.05$) increased the growth and yield characteristics of lettuce (Table 4) as well as the percentage N, P and K contents of the lettuce leaves (Table 5) in comparison to the sole RP and the control treatments. The amount of available P in the soil positively related to the plant height (R^2=63), leaf area (R^2=0.55), dry weight (R^2=0.73) and the percentage P in the leaf (R^2=0.88) of lettuce. The PM at 2.5gRP + 5gPM recorded the highest significant ($P<0.05$) values of the parameters than the other treatments, bearing the same trend as the available P released in the soil discussed above.

Table 4. Growth and yield parameters of lettuce after soil amendment

Treatment (per kg soil)	Plant Height (cm)	Leaf Area (cm²)	Dry weight (g)
Control	9.20	38.18	11.36
2.5g RP	11.90	40.50	12.23
2.5g CM	12.30	49.02	14.11
2.5gRP + 2.5g CM	15.70	79.75	16.58
2.5gRP + 5gCM	15.70	75.46	17.53
2.5gPM	16.70	75.40	18.12
2.5gRP + 2.5gPM	19.50	78.02	19.21
2.5gRP + 5gPM	21.30	80.80	23.62
2.5gPG	13.10	59.64	14.51
2.5gRP + 2.5gPG	13.60	65.28	15.40
2.5gRP + 5gPG	15.55	69.84	16.62
LSD	1.51	2.44	1.60
CV	4.50	12.00	9.70

Table 5. Nutrient content of lettuce after soil amendment

Treatment (per kg soil)	N (%)	P (%)	K (%)
Control	1.40	0.37	1.23
2.5g RP	1.54	0.40	1.97
2.5g CM	1.75	0.40	1.28
2.5gRP + 2.5g CM	1.88	0.44	1.64
2.5gRP + 5gCM	1.90	0.48	1.91
2.5gPM	1.71	0.43	2.00
2.5gRP + 2.5gPM	1.93	0.50	1.34
2.5gRP + 5gPM	2.03	0.56	2.03
2.5gPG	1.88	0.42	1.80
2.5gRP + 2.5gPG	1.89	0.45	1.82
2.5gRP + 5gPG	1.92	0.47	1.68
LSD	0.77	0.10	0.85
CV	3.22	5.10	2.00

Conclusion

The application of poultry manure, cattle manure and pig manure in rock phosphate soil amendment improved the dissolution of P from the rock phosphate, with the poultry manure being much more effective. The dissolution increased with the quantity of manure applied. The growth, yield and percentage P content of lettuce increased relative to the available P in the amended soil. Repetition of the experiment using nuclear techniques is imperative as this will give more accurate results.

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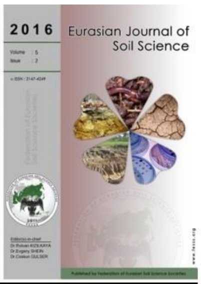

Optimization of sodium extraction from soil by using a central composite design (CCD) and determination of soil sodium content by ion selective electrodes

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Abstract

Rapid determination of sodium (Na) ions in soil samples using ion selective electrodes (ISE) was investigated in this study. The compatibility of ISEs with soil extraction solution is a challenging subject as various effects such as pH, ionic strength and other interferences have to be considered as well as efficiency of the extraction solution. Because almost every type of sodium salt is soluble in water, and the pH of water is suitable for ISE studies, it was chosen as the soil extractant. Firstly, the extraction parameters were optimized by using a central composite design (CCD), secondly thirty agricultural soil samples were extracted with water and the extracts were measured by Na-ISE in a previously developed flow system. The results were compared with ion chromatography (IC) as the reference method, and the regression analysis between IC and ISE results yielded a high correlation (R^2 = 0.9408). It was concluded that, ion selective electrodes can be used with water as an extraction solution for rapid determination of sodium in soil samples.

Keywords: Central composite design (CCD), ion selective electrodes (ISE), soil analysis, sodium

Introduction

In history, countless civilizations that began in the irrigated agriculture areas became extinct due to soil salinity. Even today, despite the state-of-the-art techniques in soil and farm management, non-agricultural lands have become quite common due to salinity. Today more than ever salinity is one of the most important factors threatening the sustainability of irrigated agriculture. If not brought under control salinity reduces the productivity, and in many cases lands may become entirely non-productive (Kanber and Unlu 2010).

Soil salinity accrues from accumulation of water soluble salts or retention of exchangeable sodium in high levels by adsorption complex in soil. Soils that are exposed to various types and levels of salinity contain salts formed by Na^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}, K^+, HCO_3^-, CO_3^{2-}, NO_3^- ions (Eruz, 1979). Sodium is one of the most commonly found elements in nature. It abounds in soil, water and air (Kacar, 2012). Sodium exists as an exchangeable cation (Na^+) held on soil colloids and as a Na^+ ion in the soil solution (Benton and Jones 2001). Even though sodium is not a major plant food nutrient, it has a critical role in soil health. Excessive amounts of sodium ruins the physical structure of soil. The aggregates fragmentize by causing decline in water and air permeability of soil, adversely affecting root development. As structure deteriorates, stickiness of soil increases making cultivation impossible. Sodium content of agricultural soils varies between 0.1 - 1 %, 0.63 % is average. In general, the salinity of the soil in arid and semi-arid regions is higher than the soil in rainy regions (Kacar, 2012). When NaCI content of soil exceeds 0.5 %, salinity, problem shows up (Kanber and

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Unlu, 2010). Sodium content of soil is conventionally determined by flame photometer. However, conventional soil testing methods are costly and time consuming processes, also the number of samples that can be analyzed are limited. Drawbacks in conventional soil analysis methods have led to the investigation of fast in-field monitoring systems. Ion-selective electrode (ISE) technology is a good alternative in this sense as it is an accurate, fast, economic and sensitive method. Above all, ISEs are portable enabling fast and in-situ soil analysis. Many of ISEs are commercially available for use in various matrixes, and significant developments have been made in ISE technology in recent years by development of various ion-selective membranes including NO_3, K, Ca, and Na which are the most important soil nutrients, and also many researches have been working on developing phosphate ion-selective electrodes (Tsukada et al. 1989; Carey and Riggan 1994; Knoll et al. 1994; Xiao et al. 1995; Chen et al. 1997; Wroblewski et al. 2000; Bratovcic et al. 2009; Eren ve ark., 2014).

The selection of a convenient extraction solution is the key point of ISE studies, especially in difficult matrixes like soil. The extraction solution should be compatible with ISEs while extracting the target ions from soil efficiently. Many solutions are available for conventional soil analysis as extractant, but only few of them can be used with ISEs successfully because of their high ionic strength, high acidity or alkali (Wang and Scott, 2001; Wang et al. 2004; Zbiral and Nemec, 2005; Ciesla et al. 2007; Madurapperuma et al. 2008; Matula, 2009; Bortolon and Gianello, 2010; Kahveci and Atalay, 2010; Bortolon et al. 2011). In this study, water was chosen as soil extractant, because almost every sodium salt is soluble in water, and also pH of water is suitable for ISE studies.

The focus of this research was to investigate the applicability of soil sodium content measurement by using ion selective electrode technology. Response surface methodology (RSM) is a group of mathematical and statistical techniques that enables modeling and analysis of problems by assessing influences of each input variable on a particular response and determining optimum values for this response (Montgomery, 1997; Khuri and Mukhopadhyay, 2010). Extraction conditions of soil sodium was optimized by using RSM and Central Composite Design (CCD). The extraction parameters were determined as soil:extractant ratio (w:v), shaking time (min) and shaking speed (rpm). CCD is appropriate for fitting a quadratic mathematical model and it is used to optimize the effective input variables with a minimum number of experiments, also enabling analysis of the interaction between the parameters. In the sequel, Na-ISE measurement results were compared with ion chromatography (IC) results as the reference method.

Material and Methods

Measurement system

Tests of the Na-ISE were conducted with a previously developed flow system (Figure 1) that contains a commercially available all solid state sodium ion selective electrode, a measuring cell into which the electrodes (Na-ISE and reference electrode) were inserted, containers to hold solutions (1 and 2), and a control unit that allows collection of voltage data from Na-ISE measured relative to the reference electrode (R). Each test began with the flow of 0.01 mol/L $CuSO_4$ baseline solution. The desired volume of test solution was fed to the flow line by using 3-way mini valve as shown on the scheme (Figure 1) and measurements consistently continued under computer software control. When the test solution sensed by the detector (Na-ISE), voltage differences occurred by forming peaks with different heights according to the ion concentration. After the system reached equilibrium by baseline solution, each individual test solution was introduced to the flow line. The electrode was first calibrated with $1x10^{-5}$, $5x10^{-5}$, $2.5x10^{-4}$, $1.25x10^{-3}$, $6.25x10^{-3}$ mg/L NaCl solutions prepared in water.

Optimization of extraction parameters

Water, which is compatible with ion selective electrodes, was used to extract Na ions from soil. Extraction was done by adding the extractant into the soil sample and shaking the mixture in an orbital shaker. The parameters that effect the extraction efficiency were determined as soil:extractant ratio, shaking time and shaking speed. CCD was used to optimize the extraction parameters. In order to determine the relationship between the extraction parameters and obtained sodium concentration, the data must be statistically analyzed by using regression equations (Montgomery, 1997). For regression equation, the extraction parameters were coded as shown in Table 1.

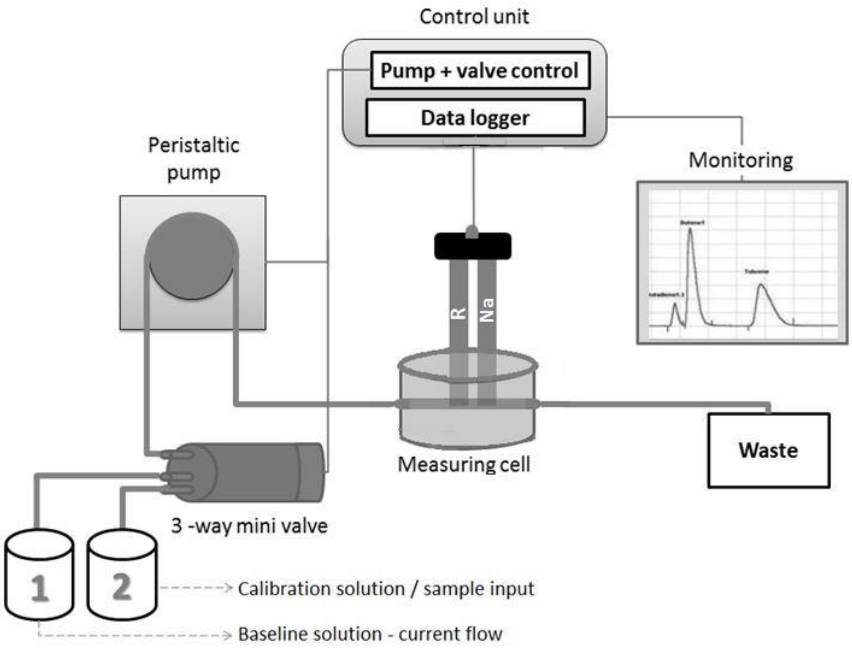

Figure 1. The scheme of the flow system that uses Na ion selective electrode as detector.

Table 1. Coded values of central composite design (CCD) (Liu et al. 2006)

Parameters	Coded Parameters	Coded Levels				
		-2	-1	0	1	2
Soil: Extractant ratio (w/v)	X_1	1:1	1:2	1:3	1:4	1:5
Shaking time (min)	X_2	10	20	30	40	50
Shaking speed (rpm)	X_3	150	180	210	240	270

A central composite design (CCD) with three factors, i.e., soil: extractant ratio (w/v), shaking time (min), and shaking speed (rpm), was adopted in the present study. The experimental design consisted of three variables with five levels i.e., low (-2) and high (+2) with 20 experimental runs. The experimental runs and variables are shown in Table 2. The experiments were randomly conducted in order to reduce the effects of uncontrolled factors. The soil sample which was chosen for optimization experiments had a sandy loam texture (% 63.03 sand, % 21.14 silt, % 15.83 clay) and its pH and EC were 5.80 and 0.13 dS/m (1:1 w/v), respectively.

Sodium extraction parameters (X_1, X_2, X_3) were correlated with the Na-ISE responses according to a quadratic model. Experimental data was analyzed by using analysis of variance (ANOVA) to determine the effects of each variable in quadratic equation. Thus, variables with small effect ($p<0.05$) were eliminated from the quadratic equation and the final mathematical model was obtained. The process of optimization was performed by validating the mathematical model to obtain maximum Na-ISE response using Mathcad software (Mathcad Prime 3.0). All three dimensional response surface graphs were generated using Statistica software, (Statsoft). All reagents were prepared by using ultrapure water obtained with a Milli Q system (Millipore Direct Q-UV3). All chemicals with analytical grade were purchased from Merck (Merck KGaG, Darmstadt, Germany). Stuart SS11 orbital shaker was used in extraction process.

Comparison of ISE with IC

The new extraction method needed to be tested with a variety of soils to represent the extreme features of agricultural soils in Turkey. For this purpose, thirty soil samples were provided from Soil, Fertilizer and Water Resources Central Research Institute in Ankara. The properties of thirty soil samples are given in Table 3.

Table 2. Central composite design (CCD) (Liu et al. 2006)

Experiment No	Coded Parameters			Actual Parameters		
	X_1	X_2	X_3	Soil: Extractant ratio (w/v), X_1	Shaking time (min), X_2	Shaking speed (rpm), X_3
1	-1	-1	-1	1:2	20	180
2	-1	1	1	1:2	40	240
3	1	-1	1	1:4	20	240
4	1	1	-1	1:4	40	180
5	-1	-1	1	1:2	20	240
6	-1	1	-1	1:2	40	180
7	1	-1	-1	1:4	20	180
8	1	1	1	1:4	40	240
9	-2	0	0	1:1	30	210
10	2	0	0	1:5	30	210
11	0	-2	0	1:3	10	210
12	0	2	0	1:3	50	210
13	0	0	-2	1:3	30	150
14	0	0	2	1:3	30	270
15	0	0	0	1:3	30	210
16	0	0	0	1:3	30	210
17	0	0	0	1:3	30	210
18	0	0	0	1:3	30	210
19	0	0	0	1:3	30	210
20	0	0	0	1:3	30	210

Table 3. Chemical and physical characteristics of 30 soil samples used in this study.

ID	pH	EC, dS/m	Textural class	ID	pH	EC , dS/m	Textural class	ID	pH	EC , dS/m	Textural class
1	8.25	0.43	Heavy Clay	11	8.55	0.29	Sandy	21	7.71	0.49	Loam
2	8.02	0.45	Clay	12	7.85	0.58	Loam	22	7.88	0.41	Loam
3	7.98	0.47	Silty Clay	13	8.22	0.94	Loam	23	6.78	0.36	Loam
4	7.87	0.39	Silty Clay Loam	14	8.17	0.38	Clay Loam	24	7.70	0.33	Clay
5	7.82	0.64	Clay Loam	15	8.05	0.44	-	25	4.82	0.08	Sandy
6	7.85	0.86	Loam	16	8.10	0.51	-	26	6.41	0.40	Clay Loam
7	8.01	0.55	Sandy Clay	17	7.83	0.62	Loam	27	7.32	2.61	Heavy Clay
8	7.96	0.42	Sandy Clay Loam	18	8.00	0.51	Loam	28	7.72	0.41	Loam
9	7.9	0.62	Sandy Loam	19	7.75	0.67	-	29	8.03	0.28	Loam
10	7.33	0.34	Loamy Sand	20	7.6	0.62	Sandy	30	8.14	0.42	Loam

NOTE: pH and EC measurements; 1:1 w/v, 25 ^{0}C.

The soil samples were extracted with water at optimum conditions, which were previously determined by CCD and RSM approach as 1:4.7 soil:extractant ratio (w:v), mixing at 216 rpm for 23 minutes in an orbital shaker. The extracted samples were filtered with Whatman No. 42 filter paper. Every extract was analyzed with Na-ISE in the flow system, and with ion chromatography (IC) as a reference method. The IC analyses were held in Soil Water Laboratory in Atatürk Soil, Water and Agricultural Meteorology Research Station Management.

Results and Discussion

Optimization of extraction parameters

The experimental data of CCD model was analyzed by the response surface regression procedure to fit the quadratic model, providing regression coefficients. The ANOVA for the quadratic model for Na concentration is listed in Table 4.

Table 4. Statistical parameters obtained from the analysis of variances (ANOVA) for Na ions concentration

Regression Statistics	
Multiple R	0.94
R^2	0.89
R^2 adjusted	0.78
Standard Deviation	1.09

The model multiple R observed was 0.94 stating that the model was significant. The quadratic regression model for sodium concentration (Y) can be represented by the following Equation (1):

$$Y=9.66+(2.20)*X_1+(0.4)*X_2+(0.03)*X_3+(0.50)*X_1*X_1(0.06)*X_2*X_2-(0.31)*X_3*X_3-(0.26)*X_1*X_2-(0.26)*X_1*X_3+(0.12)*X_2*X_3 \quad \text{Equation (1)}$$

Here in this equation the coefficient with second order term shows the quadratic effect. Positive sign in front of the terms indicates that this variable is proportional to Na-ISE response while negative sign means the variable is inversely proportional to Na-ISE response. The model also yielded high correlation with the actual results, R^2 = 0.94, shown in Figure 2.

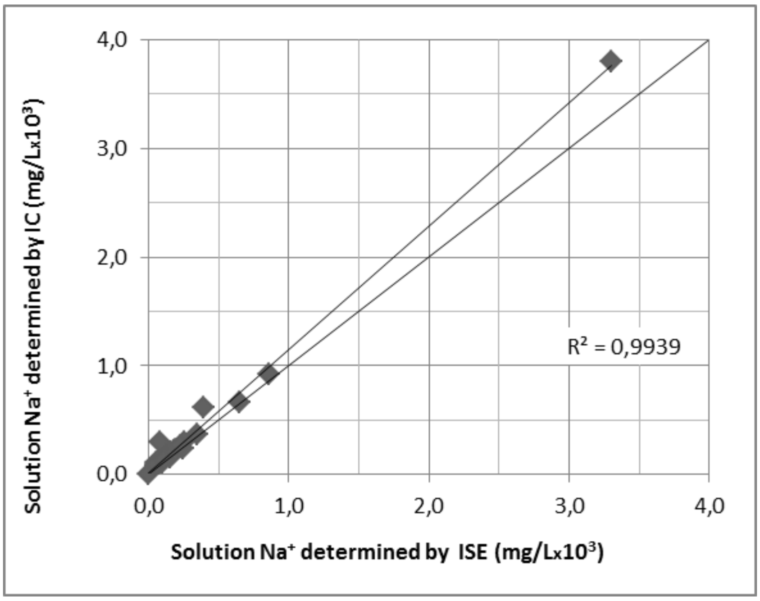

Figure 2. Comparison of the mathematical model with the actual results

The three dimensional response surface graphs that reveal the relation between the extraction parameters and Na^+ concentration were generated by Matlab software, and are shown in Figure 3. Both the graphs and mathematical model indicate that soil:extractant ratio effects Na^+ ion results the most.

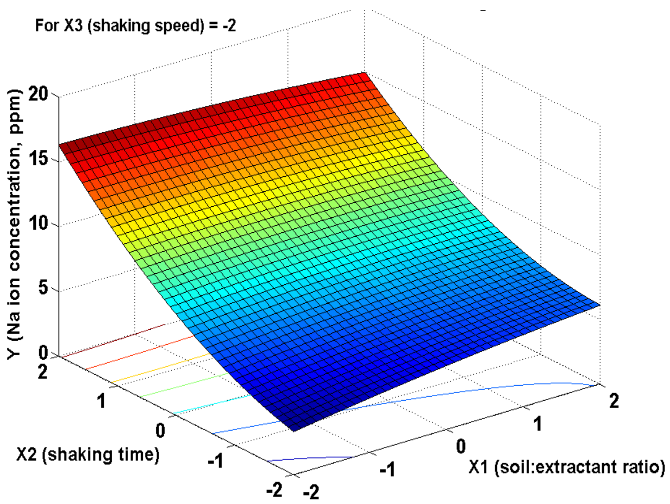

Figure 3. Effects of shaking time and soil:extractant ratio on Na-ISE response while shaking speed is minimum (X3 = -2).

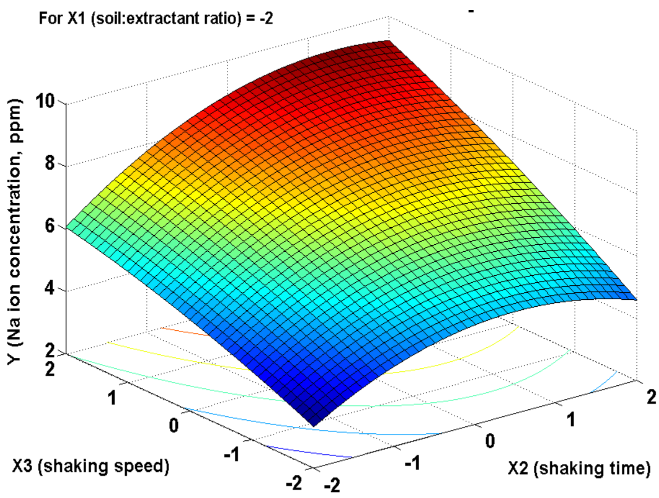

Figure 4. Effects of shaking speed and shaking time on Na-ISE response while soil:extractant ratio is minimum (X1 = -2).

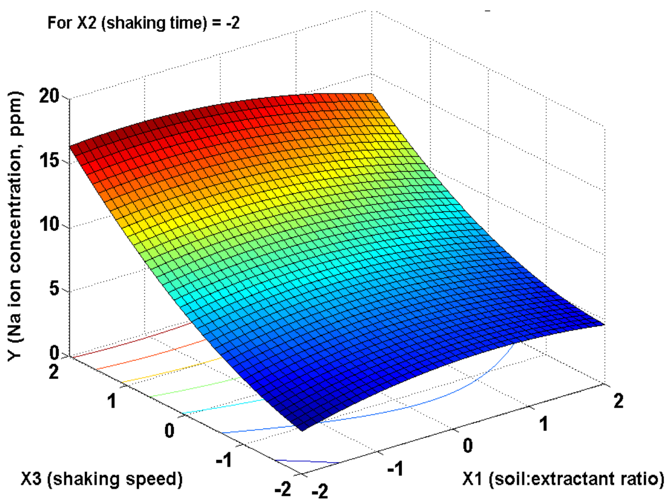

Figure 5. Effects of soil:extractant ratio and shaking speed on Na-ISE response while shaking time is minimum (X2 = -2).

The process parameters were optimized in Mathcad by using the mathematical model and targeting the maximum Na-ISE response. The optimum extraction conditions are listed in Table 6.

Table 5. Optimum extraction conditions determined by Mathcad

Extraction Parameter	Optimum Value
Soil: Extractant ratio (w/v)	1:3
Shaking time (min)	15
Shaking speed (rpm)	260

Comparison of ISE with IC

The primary aim of the research is to develop a sodium ions measuring method based on ion selective electrodes (ISE), so the newly developed soil extraction procedure was applied to the sensor system and the results were compared with those obtained from IC as the reference method. Regression analysis that were applied to ISE and IC results yielded high correlation of R^2 = 0.9408 (Figure 3). Na-ISE used with water provided close agreement with IC analysis.

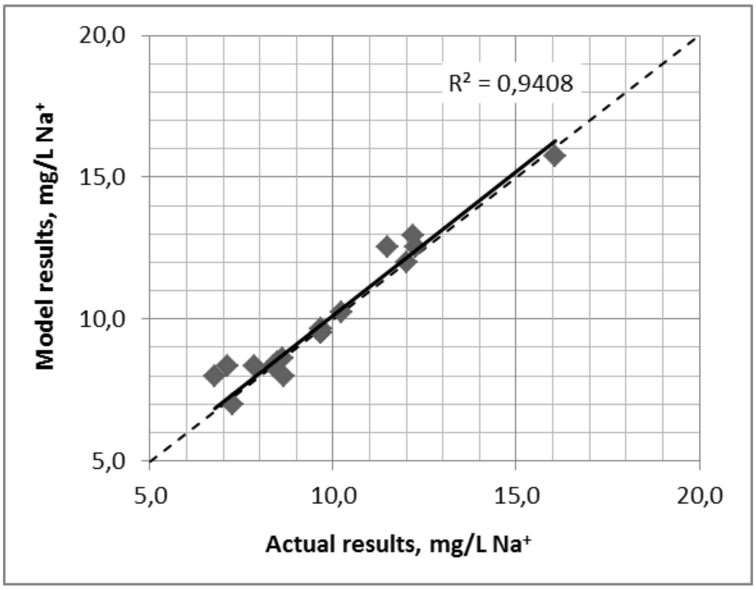

Figure 6. Relationship between soil extract Na^+ determined by ISE and IC.

Conclusion

A new analysis method was developed in order to determine Na^+ ion concentration in soil extracts. The extraction procedure was determined by optimizing the extraction conditions using central composite design (CCD) and RSM, this new method was tested with 30 different agricultural soil samples from Turkey. The comparison of Na-ISE and IC results yielded high correlation (R^2 = 0.9408) with almost 1:1 relationship, which indicates that Na measurement can be performed by Na-ISE in this flow system together with water extraction, at least for 30 soil samples examined in this study. Additional research and tests with greater number of soil samples are needed to further validate the applicability of these results.

Acknowledgements

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Heavy metal pollution affected by human activities and different land-use in urban topsoil: A case study in Rafsanjan city, Kerman province, Iran

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Abstract

The excessive input of trace elements into urban soil has become one of the most important concerns in industrial and crowded cities all over the world. The contamination of urban soils can affect the health of people living in urban areas, and the surrounding ecosystems. Current study was conducted to assess the effects of human activities as well as different land-use on accumulation of trace elements in urban topsoil and also identify the potential risks to human health in Rafsanjan (Iran). A total of 100 topsoil samples were taken from different localities of Rafsanjan City and analyzed for Zn, Pb, Cu and Cr using the atomic absorption spectrophotometric method. Pollution index (PI) was calculated for each trace element to identify the rate of trace element accumulation with respect to the background values. Land-use map and geochemical maps were also created for evaluating of spatial distribution of pollution index and trace elements concentration in the studied area. Overlapping the concentrations map and land-use map revealed that the highest values of pollution index and trace elements concentration were located in central part of the city and highways with a great vehicle traffic load and also in the vicinity of industrial factories that increased potential health hazards to the local community. On the other hand, lowest values of trace elements were located in green-lands with strict vehicle traffic laws. These results indicated that different land-use and human activities have affected quality of urban topsoil of Rafsanjan resulting in great apprehensions regarding public health in crowded parts of the city.

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Introduction

Trace elements are ubiquitous in our environment, as a result of natural and anthropogenic activities, and humans are exposed to them through various pathways such as inhalation of dust (Christoforidis and Stamatis, 2009; Narouzi and Khademi, 2015), food chain (Harmanescu et al., 2011), etc. Some trace elements such as Zn and Cu are necessary due to their role as metalloenzymes (Harmanescu et al., 2011). According to Food and Drug Administration (FDA) these metals are known as cofactors of a number of enzymes (FDA,

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2001); however, recommended dietary allowances (RDA) of Zn and Cu for adults are 8–11 and 0.9 mg day^{-1}, respectively, and beyond this range deficiency and toxic effects are observed (FDA 2001; Singh and Garg, 2006). Cu surplus had been associated with liver damage (FDA, 2001). Furthermore, metal fumes cause fever with flu-like symptoms; also, hair and skin discoloration may be caused by fumes of this trace element, although dermatitis has not been reported. Systematically as well, copper dust and fumes may cause irritation of the upper respiratory tract, a metallic taste in the nausea and mouth (Broyer et al., 1972).

The clinical signs of zinc toxicities have been reported as vomiting, diarrhea, bloody urine, icterus, liver failure, kidney failure and anemia (Fosmire, 1990). Also, Zn reduces immune function and the levels of high density lipoproteins (FDA, 2001). Lead is considered to be the most important toxin of trace elements which can be absorbed through ingestion by food and water, and inhalation (Duruibe et al., 2007). Lead poisoning may cause inhibition of the synthesis of hemoglobin; joints and reproductive systems, dysfunctions in the kidneys, cardiovascular system and acute and chronic damage to the central nervous system as well as peripheral nervous system (Ogwuegbu and Muhanga, 2005). Based on the previous reports lead poisoning is also associated with damage to the gastrointestinal tract and urinary tract which result in bloody urine, neurological disorder and can cause severe and permanent brain damage (INECAR, 2000). Poor development of the grey matter of the children's brain is caused by lead thereby resulting in poor intelligence quotient (IQ) (Udedi, 2003). Acute and chronic effects of lead result in psychosis (Duruibe et al., 2007).

Chromium is also one of the known environmental toxic pollutants in the world. Despite a lot of research on the role of chromium in biological systems, it is still a controversial issue and no significant conclusions can be arrived with the available data. Chromium (VI) enters into the cells readily and is reduced to form stable chromium (III) complexes which react slowly and were considered non-toxic. Nevertheless, recent studies suggest that chromium (III) in fact damages cellular organelles, DNA and proteins and can be lethal to organisms and their offspring (Natesan and Balachandran Unni, 2008).Chromium exposure can cause skin rashes, respiratory problems, kidney and liver damage, upset stomach, ulcers, alteration of genetic material, weakened immune systems, lung cancer and ultimately death (McGrath and Smith, 1990; Kabata-Pendias and Pendias, 2011).

In spite of our comprehensive knowledge of pernicious and detrimental effects of trace elements on human health, during the last decades, contamination of urban soils by trace elements has gradually become one of the most significant concerns in metropolitan areas (Giannis et al., 2010; Minkina et al., 2014) and also impose a long-term burden on the biogeochemical cycles in the urban ecosystem (Papa et al., 2010). Extensive use of sewage sludge and waste water for irrigation, pesticide, vehicle exhausts' emissions, mining and rapid development of industries without effectual control has led to a large accumulation of trace elements in soil (Shi et al., 2009).

Street dusts and top roadside soils in urban areas considered as asign of trace element pollution from atmospheric deposition (Christoforidis and Stamatis, 2009). Although leaded gasoline is the main source of Pb, other trace elements such as Cu, Zn and Cd are coming from car components, tire abrasion, lubricants, industrial and incinerator emissions (Markus and McBratney, 1996; Wilcke et al., 1998). It is proved that source of Cr in the street dust is corrosion of cars (Ferguson and Kim, 1991; Akhter and Madany, 1993) and plating of some motor vehicle parts (Al-Shayep and Seaward, 2001), respectively.

Comparing the trace element concentration with guideline or quantifying of an accumulation factor (pollution index, PI) with respect to the background concentration issued to evaluate the degree of contamination in the urban areas (Jung, 2001). Despite the presence of numerous industrial factories as well as rapid growth of population in Rafsanjan, a few studies associated with trace elements contamination in urban topsoil have been carried out. Urban topsoil, as well as agricultural soils, is being threatened by trace elements as a result of rapid industrialization and urbanization during the last two decades. The current study, however, is focused on soil pollution by trace elements in Rafsanjan, which is attributed to vehicle emissions, domestic heating and other anthropogenic activities. The aim of the present study is to assess the effects of different land-use as well as human activities on topsoil trace element pollution and also identify the potential risk to human health in Rafsanjan.

Material and Methods

Study site

The study site is located in the urban area of Rafsanjan City in Kerman province relatively situated at the southeast of Iran (Figure 1) which is an important social economic southeast of Iran. The soil of Rafsanjan is calcareous and saline its mean annual temperature and average annual precipitation are 14.5 °C and 140 mm, respectively, with a population of approximately 0.4 million.

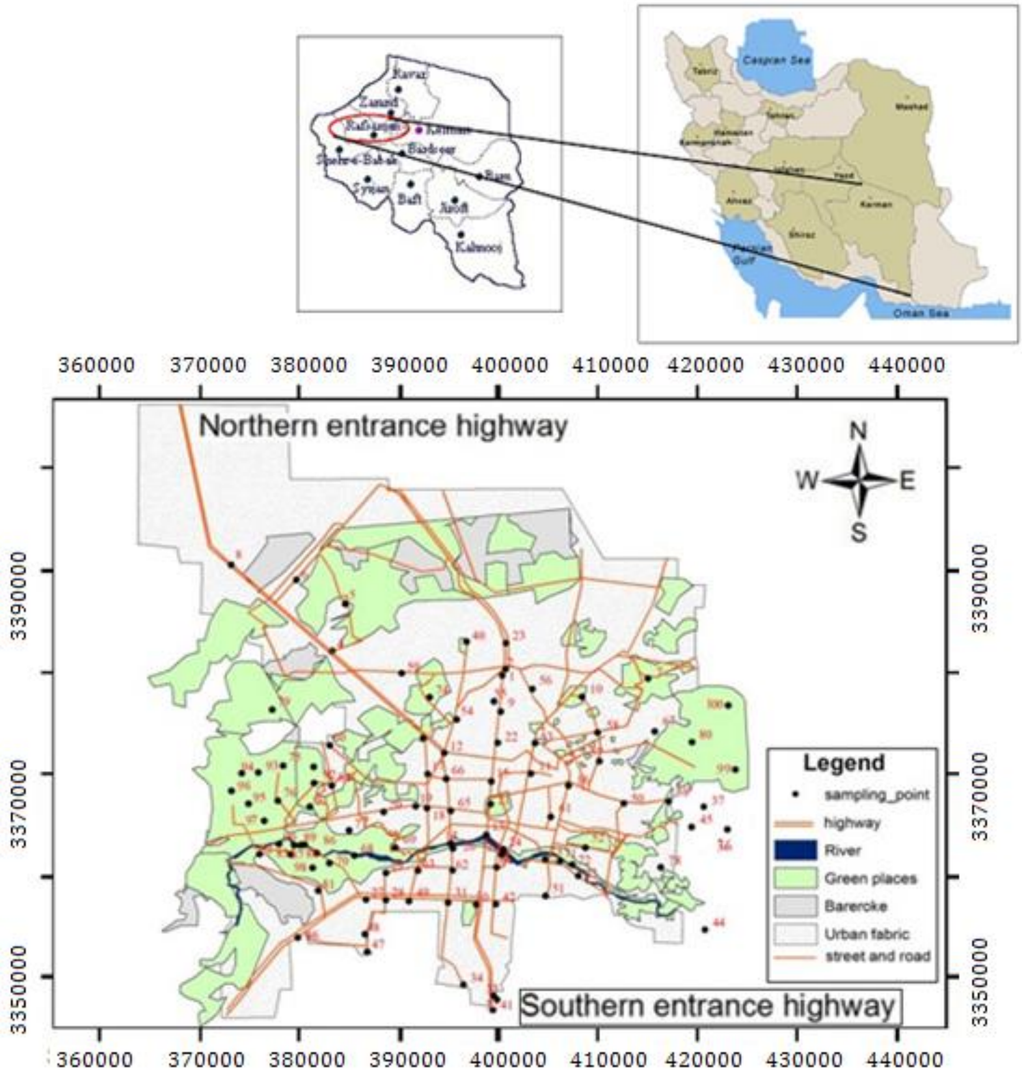


Figure 1. Studied area, sampling points and land-use map of Rafsanjan

Sampling

Sampling points were chosen from different parts of Rafsanjan extending from longitude55˚, 59', 30"E and latitude 31˚, 13"N. For this topsoil investigation, uppermost layer of the soil profile (0–1 cm), where industrial dust and emissions from vehicles are deposited, was collected. This indicates that these topsoil textures are characterized by the predominance of finest particle size. Also the background soil samples were collected from 60cm depth (Morton-Bermea et al., 2009). Sampling points were selected from different land-use to cover the different zones such as parks, green-lands, road side, crowded places and industrial areas of the city in order to specify and clarify the effects of green spaces or urbanized points on polluting the surface and comparing several points with different usage.

A total of 100 samples were collected in summer 2013, the top 1 cm layer of the soil profile was taken with a plastic trowel, stored in a plastic bag and transferred to laboratory immediately. The coordinates of the sample locations were recorded with a GPS, and the sampling points locations are given in Figure 1.

Laboratory analysis

Samples were air-dried. About 25 g portions were ground until fine particles (sieved by 200 µm nylon mesh) were obtained (Morton-Bermea et al., 2009). In order to measure the trace elements' concentrations (Pb, Zn, Cu, Cr), 2.5 g of each sample were digested by nitric acid (4 M) in water bath at 80 °C (Sposito et al., 1982). Afterward, trace elements' concentrations were determined by atomic absorption spectrophotometer Perkin-Elmer 3030.

Statistical analysis and pollution index calculation

Descriptive statistics including the mean, standard deviation, minimum, maximum, median and range were determined and PI was defined as trace element concentration in the sample (topsoil)/background median value. Analysis of the data was done with SPSS statistical software v 16.

Geo-statistical analysis based on GIS

Spatial interpolation and GIS mapping (kriging) techniques were employed to produce spatial distribution maps for the four observed trace elements by ArcGIS v.9. (Tao, 1995; Cattle et al., 2002; Yasrebi et al., 2009). Regarded as mirror of pollution rate in surface soil compared to background, pollution index (PI) was used for depicting geochemical maps. The PI was defined as the value of the pollution index of each element, calculated by the use of the ratio of the trace element concentration in topsoil to the background concentration of the corresponding trace element as the following formulation (Lu et al., 2008; Faiz et al., 2009; Wei et al., 2009; Morton-Bermea et al., 2009).

$$PI = \frac{Ci}{Bi}$$

Where C_i is the concentration of element in topsoil, B_i is the background value.

Results and Discussion

Land-use study

As shown in Figure 1, most of the green-lands are located in west, southwest, east and also river bank, while the gigantic highways which connect the north of Iran to south are located in northwest, north and south. The northwest of the city is also considered as the greatest industrial area of Rafsanjan city. Most of the urban fabrics, traffic load and commercial centers are seen in central parts of Rafsanjan.

Statistical study

Table 1 represents minimum, maximum, median and standard deviations for each trace element and intervention guideline, dictated by Canadian Council of Ministers of the Environment (CCME, 2007).

Table 1. Minimum, maximum, median, mean, standard deviations and maximum acceptable concentration (MAC) of each analyzed trace elements in samples and background

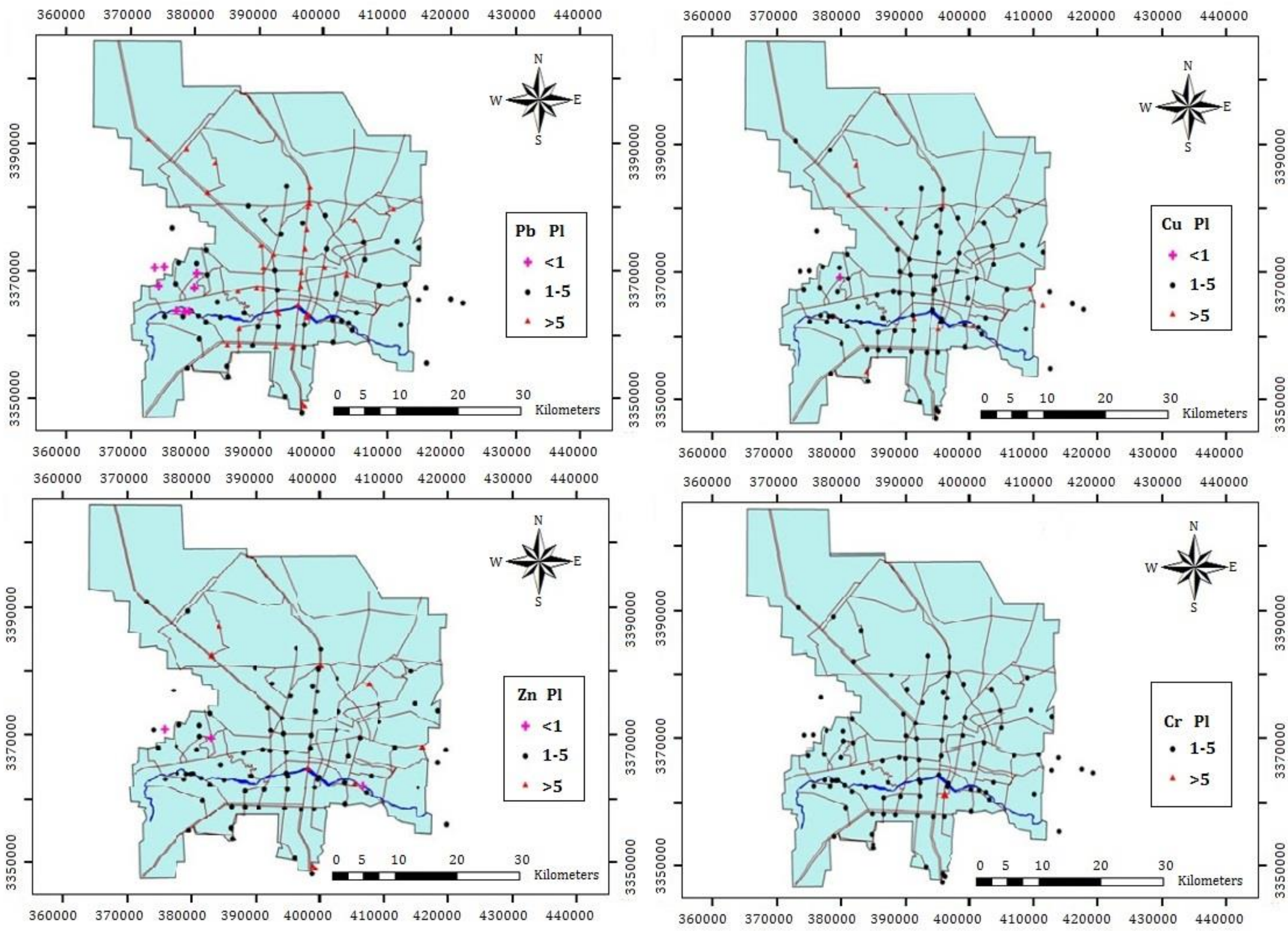
	Median (mg kg^{-1})	Mean (mg kg^{-1})	Min [a] (mg kg^{-1})	Max [b] (mg kg^{-1})	MAC [c] (mg kg^{-1})	Range	SD [d]
Samples							
Pb	73.2	84.2	17.4	215.4	140	198	50.9
Zn	154.8	196.7	38.4	651.6	200	613.2	118.6
Cu	52	59.9	20.8	144	63	123.2	30.4
Cr	59	61.1	29.5	110	64	80.5	16.7
Background							
Pb	19.9	25.1	23.1	27.6		4.5	3.25
Zn	63.6	86.2	64.3	99.1		34.8	17.40
Cu	21.2	24.2	17.9	28.5		10.6	4.51
Cr	21.1	23.3	18.3	26.4		8.1	3.43

[a] Minimum concentration (mg kg^{-1})
[b] Maximum concentration (mg kg^{-1})
[c] Maximum acceptable concentration (mg kg^{-1})
[d] Standard deviation

Based on the obtained results, maximum values of Zn, Pb, Cu and Cr were 651.6, 215.4, 144 and 110 mg kg^{-1}, respectively, while minimums were 38.4, 17.4, 20.8 and 29.5 mg kg^{-1}. Maximum values of all studied trace elements in samples were higher than the selected standards. With respect to Zn, Pb, Cu and Cr concentrations, about 36.84, 15.79, 38.95 and 40 % of data were larger than maximum acceptable concentration (MAC). None of the means, however, was larger than MAC. Mean concentrations of the analyzed trace elements in urban soils were196.7, 84.2, 59.9 and 61.1 mg kg^{-1}, respectively, for Zn, Pb, Cu and Cr, which exceed their background values in all sites. Concentration ranges of Zn, Pb, Cu and Cr were observed to be 613.2, 198, 123.2 and 80.5 mg kg^{-1}, respectively (Table 1). Zinc showed the highest range of concentration while the lowest range belonged to chromium. Medians as well as mean values for the all trace elements were more than background. Wei and Yang (2010) reported that concentration of trace elements in urban soils of all studied cities in China exceed their background values. It can be attributed to the role of human activities in increasing trace element concentrations (Morton-Bermea et al., 2009).

Pollution index and geochemical maps

Figures 2 and 3 represent the values and limitations of four evaluated trace elements in several sampling points based on the PI values and concentration of them. The PI was classified as: PI <1 low level of pollution; 1< PI < 5 moderate level of pollution and 5 < PI high level of pollution (Morton-Bermea et al., 2009).



(a) (b)

Figure 2. (a) Pollution index (PI) values for Pb and Zn in the studied zone. PI values were calculated as ratio between sample metal concentration and background median. (b) Pollution index (PI) values for Cu and Cr in the studied zone. PI values were calculated as ratio between sample metal concentration and background median

In comparison with the other trace elements, Pb allocated the largest value of PI to itself, varied from 0.9 to10.8.All sampling points with high level of pollution (PI > 5) are located in the center, south and northwest of the city, where the highest amount of vehicle traffic load and industrial activities can be seen. Other scientists had also reported that maximum amounts of lead were measured in central parts of cities (Hernandez-Alvarez 2001; STV Gobierno del Distrito Federal 2008; Morton-Bermea et al., 2009). Incontrovertibly, lead has been partly derived from tetraethyl lead, used as an additive to gasoline, released by vehicles into the environments so that more urban areas in the vicinity of highways have a critical lead concentration in topsoil. As shown in Figure 3, the highest concentration of Pb was also measured in northwest, north and central part of the city where human health is threatened by severe symptoms including anemia, a decrease handgrip strength, pale skin, abdominal pain, nausea and paralysis of the wrist joint, etc. On the other hand, continued exposure can lead to decreased fertility and/or increased risk of miscarriage or birth defects (Zheljazkov and Jekov, 1994).

It is obvious that minimum amounts of PI and concentration of Pb were found in the west and south west of Rafsanjan. Based on Figure 1 these parts of the city belong to green-lands and river bank with strict traffic rules. Studying some of China's urban topsoil and road dusts, Shi et al. (2008) reported that traffic contaminations are one of the major sources of trace elements in urban environment.

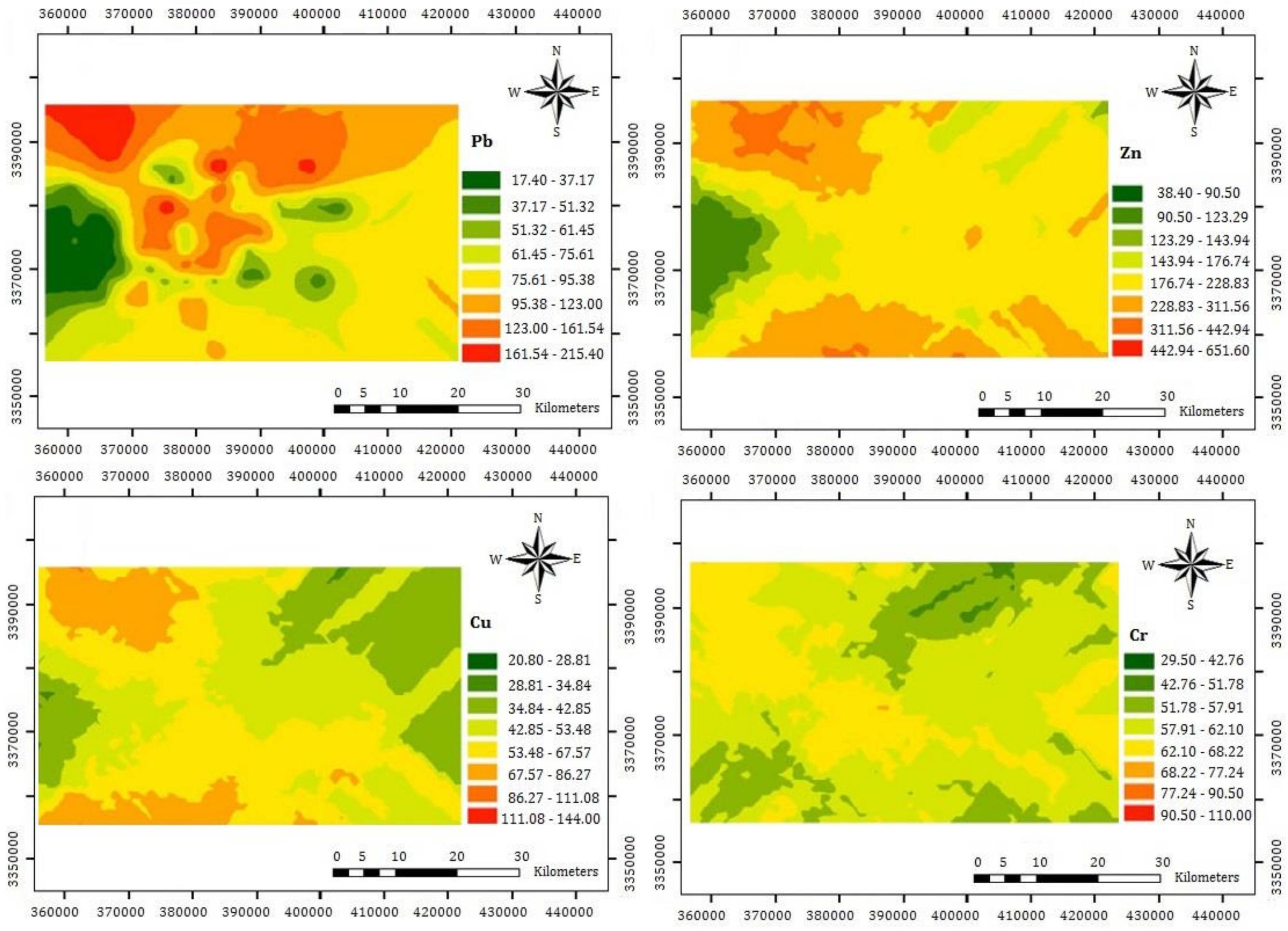


Figure 3. Spatial distribution of trace elements (mg kg^{-1}) in the studied zone

PI values for Zn in different urban topsoil varied from 0.6 to 10.2. Maximum values of PI for Zn are found to be in south, north and northwest. Although the number of PIs which is classified in a high level of pollution (PI > 5) is less than those for lead, comparison between Figures 1 and 3 revealed that the maximum concentration of Zn was measured in the vicinity of industrial factories located in north and northwest, and also the north and south entrance highways of Rafsanjan. Lower Zn concentration in center compared to the north and northwest suggests that concentration of Zn in topsoil is more relative to the industrial activity than vehicle traffic load. Nevertheless, concentrations of zinc in the central parts of Rafsanjan with high traffic load were higher than its concentration in the west and southwest (dense green-lands), which can prove the role of vehicles' emissions or abrasion to increase concentration of Zn. Despite the fact that Zn is an essential element for plants, animals and human, prolonged exposure to this metal in the contaminated part of the city may cause vomiting, icterus, bloody urine, kidney failure, anemia and liver failure, etc.

(Fosmire, 1990). Similarly to lead, lowest concentrations of Zn were also found in the west and southwest of Rafsanjan, which reminds the role of green-lands and restrictive rules of traffic. Many scientists believe that the sources of Pb, Zn, Cu and Cr in urban areas are mainly derived from industrial activities and traffic emission (Ferguson and Kim, 1991; Akhter and Madany, 1993; Markus and McBratney, 1996; Wilcke et al., 1998).

PI values obtained for Cu and Cr are more stable with the ranges of 0.9–6.8 and 1.4–5.2, respectively. In fact, most of the selected points have a moderate PI (1 < PI < 5) for Cu. There were just a few places in industrial and over-crowded parts of the city (north, northwest and center) that showed acute values of Cu PIs. Among the analyzed samples, only one point was found for PI <1 for Cu which, as well as other trace elements, is situated in the western green-land regarded as the lung of Rafsanjan. Figure 3 indicated that the pattern of distribution of Cu in the urban topsoil of Rafsanjan was approximately similar to that of Zn, which means risk of the liver damage (FDA, 2001), hair and skin discoloration, irritation of the upper respiratory tract, etc. (Broyer et al., 1972), are higher in north and northwest, and also north and south entrance highways of Rafsanjan than in the central parts and green places.

From Figure 2 it can be found that, there is just one point with high PI for Cr in downtown, while none of the sampling points showed low PI (PI < 1). According to Figure 3 most of the central, north and northwest areas have a concentration between 62.10 and 68.22 mg kg^{-1} which are near the MAC (64 mg kg^{-1}) and may cause respiratory problems, alteration of genetic material, weakened immune systems, etc., for humans (McGrath and Smith, 1990; Kabata-Pendias and Pendias, 2011). Lowest concentrations of Cr were measured in northeast, south and southwest where there are no significant vehicle traffic load and industrial activities as well.

Conclusion

Based on the obtained PIs, approximately all concentrations of the determined trace elements in the urban soils of Rafsanjan are higher than their background values and the maximum concentrations of Zn, Pb, Cu, and Cr in soil samples exceed the MAC. Finding the lowest quantities of analyzed trace elements in green-lands and parks with strict vehicle traffic rules on one hand and the highest amounts in highways, industrial areas and city center with highest traffic load on the other hand emphasizes the negative role of vehicles' traffic load and industrial activities in polluting the urban areas. In other words, the poor management of industrial development, urban construction and traffic load in Rafsanjan has seriously endangered the public health in most of the residential areas. It is suggested that effective monitoring of industrial activities and fuel quality as well as adoption of laws to reduce the traffic load may decrease the quantity of pollutants and trace elements' poisoning in studied areas.

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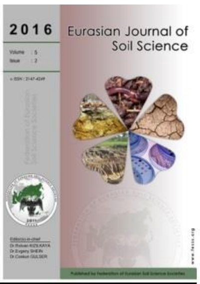

Effect of chickpea in association with *Rhizobium* to crop productivity and soil fertility

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Abstract

The growth, development and yield of chickpea (*Cicer arietinum* L.) is strongly influenced by abiotic factors such as salinity and drought in the arid conditions. The use of efficient plant growth promoting bacteria in chickpea production is the best solution to overcome those stresses. In the present study, 10 chickpea rhizobial strains were isolated and purified from the nodules of chickpea genotype grown on middle salinated soils with different chickpea cultivation histories, 3 of them were more efficient in salt tolerance and showed higher nodulation abilities. Local chickpea genotype Uzbekistan-32 was inoculated with selected *Rhizobium* bacterial strains before planting them to the field condition. Inoculation of plants with strains *Rhizobium* sp. R4, R6 and R9 significantly increased shoot, root dry matter, and nodule number by 17, 12, and 20% above the uninoculated plants, respectively. The shoot length increased by 52%, root length by 43%, shoot dry weight by 36%, and root dry weight by 64%. Inoculation significantly increased the pod number by 28% and yield up to 55% as compared to control plant. The effective indigenous rhizobial strains isolated in this study from chickpeas on middle salinated soils of Uzbekistan have the characters of broad host range, high nodulation efficiency, efficient N fixation, great salt tolerance. Soil nitrogen, phosphorus and carbon content of the soil at the end of experiments were positive in all the treatments compare control. In this study, we are focused with consideration of the relationship between chickpea and its symbiotic nitrogen-fixing root nodule bacterial strains and how it functions to influence plant productivity and soil fertility.

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Introduction

Chickpea (*Cicer arietinum* L.) is a very important legume food crop in arid regions considering as an essential source of protein, lipid, carbohydrates and vitamins for human beings. However, chickpea production is severely limited in arid areas of Uzbekistan due to soil salinity and drought condition. Also, low soil fertility and harsh climate are the important factors limiting legume production.

Uzbekistan is one of the countries most seriously affected by land degradation and desertification in the world, as is evidenced by the 85% of the land that now suffers from various levels of secondary salinization (Gintzburger et al. 2003). It limits the area under of cereals and food legumes cultivation, hence affects food security. It is further reported that approximately 20000 ha of irrigated land in Uzbekistan are lost to salinity

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and invariably abandoned every year (Toderich et al. 2009). There is urgent need to take preventive steps to overcome the current conventional agricultural approach to make agriculture in irrigated drylands sustainable. The greatest challenge to our arable agriculture in the long term is the maintenance, and preferably the improvement of soil quality.

Legume cropping systems that increase soil fertility and concurrently enhance plant productivity and prevent erosion and desertification are of major interest in many countries in the world (Egamberdieva et al. 2014). Incorporating of legume crops in crop rotation system increases the yield of both cotton and wheat which are the main crops grown in Uzbekistan occupying 80% of the total irrigated area, but crops yield have been decreasing due to worsening soil conditions (Khaitov et al. 2014).

Chickpea is usually grown on the marginal land. With the function of biological N fixation in association with rhizobial strains, chickpea could be considered as an excellent rotation and intercropping crop by improving soil fertility and structure, and decreasing soil erosion in agricultural production system (Shurigin et al. 2015).

Although, legume- *Rhizobium* symbiotic nitrogen (N) fixation is an important biological character and also the base of improving soil fertility, case in point is a lack of effective rhizobial inoculants adapting to salinated soils. Previous studies have shown that salinity and drought stress led to a significant decline in plant biomass accumulation (root and shoot), nodule development, and nitrogenase activity as well as strongly declined the yield in chickpea (Garg and Baher, 2013; Egamberdieva et al. 2014). Chickpea can restore soil fertility due to deep penetrating root system which enables them to utilize the limited available moisture (Tripathi et al. 2015).

There are evidences that certain strains of bacteria in the root nodules of leguminous crops can help tolerate toxic levels of salinity. The salt tolerance abilities of rhizobia may have an important effect on the successful *Rhizobium*-legume associations under salinity condition. It aims at obtaining ecologically safe food and higher yield without disturbance of the environment and simultaneously, improves the soil quality.

In Uzbekistan, the indigenous *Rhizobium* abundance in most soils is very low because of lack chickpea cultivation, and the rhizobial inoculants from chickpea cultivations of different regions could not adapt to salinated soils. Therefore, it is very pivotal to select efficient rhizobial strains, which are well adapted to salinated soils for developing chickpea production and improving chickpea yield and soil fertility.

In this study, a series of rhizobial strains were isolated and purified from salinated soils and local chickpea variety, and their nodulation efficiency were tested both in soil pot and field condition. Furthermore, the inoculated bacterial strains were able to persist in the rhizosphere showing colonization on root and within nodules. Present study shows that plant growth promoting rhizobacteria (PGPR) inoculation should be integrated with chickpea production program in Uzbekistan especially for marginal soils.

Material and Methods

Study site and soil sampling

Soil for pot experiment was sampled from an irrigated agricultural site located in Syrdarya Province (41°00′N, 64°00′E,) in north-eastern Uzbekistan. In these soils, cotton has been grown for the long years under a continuous monoculture production system and under flood irrigation without proper drainage facilities but using a natural flow system. According to the WRB-FAO (2006) classification, the soils of selected fields were identified as Calcisol (silt loam serozem) having a calcic horizon within 50 cm of the surface. Soil basic physical and chemical properties from this site were summarized in Table 3 and 4. The surface soil horizon was calcareous saline whereas the deeper soil horizons were only mildly alkaline. The orchic horizon is low in organic matter. The climate is semiarid with mean annual air temperatures of 16^{o}C and 18^{o}C, and mean annual rainfalls of 180-200 mm. The conventional tillage consisted of moldboard plowing to 30 cm depth after harvest and offset disking, to a depth of 10 cm, prior to planting in the spring. Soil samples of 0-30 cm depth were taken with a soil corer (3.5 cm diameter). Samples were collected at the beginning March (spring), and end of the experiments July (summer). The cores were pooled; field-moist soils were sieved (<2mm) directly after collection. The soil samples were kept in black polyethylene bags and stored at 4°C. These "fresh" field-moist, sieved samples were used for the incubation study. Conventional mineral fertilizers N, P, K input rates range from 200; 140; 70 kg $ha^{-1}yr^{-1}$ respectively in all plots of the experiment.

Plant and microorganisms

Seeds of the chickpea “Uzbekistan-32” were obtained from Seed Production laboratory of Tashkent State Agrarian University. All bacterial strains were previously isolated from the rhizosphere of chickpea grown in salinated soil of Uzbekistan.

Isolation, purification, sequencing and inoculation of Rhizobial strains

Nodules from local chickpea genotype Uzbekiston-32 grown in pots filled with salinated soil brought from field sites in Sirdarya region in Uzbekistan, were used to isolate and purify rhizobial strains. There were a few years of chickpea cultivation history in Sirdarya region’s experimental field, where the soil had a higher abundance of indigenous *Rhizobium*, slightly more salinitic. The procedures of *Rhizobium* isolation and purification followed as reported in following method. First, the fresh nodules were washed in 95% ethanol for 3 min, then sterilized in 0.2% Hg_2Cl for 5 min, and rinsed with sterilized water for 5-6 times. After that, we cut the nodules in half on the sterilized slide and used a half nodule to draw lines on the surface of the acid YMA medium. Then the YMA medium was incubated upside down for 6-7 day under 28^0C. The bacterium looked like *Rhizobium* was picked to purify and obtain pure single colonies. After the gram staining, the isolated rhizobial strains were characterized based on the shape of the bacterium under the microscope.

The inoculation experiment was carried out using the specially designed big tube paper culture system. First, the filter paper with a length about 2/3 of the tube was placed into the tube. Each tube was added a 90 mL low N nutrient solution. The sealed tubes were sterilized 30 min under 121^0C.

Salt tolerance of bacterial isolates

In order to determine the optimum salt concentration for growth, bacterial strains were cultured in YEM medium supplemented with different amounts of NaCl: 2%, 3%, 4%, and 5% NaCl (w/v). The growth rate of bacteria isolates was determined with spectrophotometer after 24, 48, 72 hours.

Germination of seeds

The seeds of chickpea were first sorted to eliminate broken, small seeds and then they were surface-sterilized with a solution of 75 mL chloride + 25 mL water for 2–3 min, rinsed thoroughly with distilled water. Surface-sterilized seeds were transferred on paper tissue towels soaked in 0.5 mM $CaSO_4$ and germinated for seven days in a dark room at 25^oC.

Plant growth promotion in pots

For the seed inoculation, *Rhizobium* strains were grown overnight in TY broth. One ml of bacterial culture was pelleted by centrifugation and cell pellets were washed with 1 ml phosphate buffered saline (PBS; 20 mM sodium phosphate, 150 mM NaCl, pH 7.4) and re-suspended into PBS. The suspension used for the inoculation was adjusted to the final concentration of approximately 10^7 CFU mL^1. Uniform seedlings were first placed with sterile forceps into bacterial suspension for 15 minutes and were then transplanted into pots filled with salinated soil (500 g each pot). Two chickpea seedlings were transplanted into each pot, but later one seedling was removed. The pot experiment had two treatments: seeds without bacterial inoculation and seeds inoculated with bacterial strains. Plants were grown at 20 - 26°C during the day and 17 - 18°C at night and after six weeks the shoot and root length and dry matter of chickpea were measured.

Field experiment

The experiment was carried out on a typical salinated soil, without chickpea cultivations in its history, at the field site of experimental station Tashkent State Agrarian University, Tashkent region in Uzbekistan. The experiment was in randomized complete-block design. There were 4 treatments including 3 *Rhizobium* treatments (with inoculation R4, R6 and R9 and without inoculation- control) and local chickpea genotype Uzbekistan-32, 3 replications for each, 12 plots in total. Each plot was 10 m^2. This experiment employed the inoculants isolated from the previous tube study. These three rhizobial strains had the highest nitrogenase activities and used peat and plant ash mixture (1:1, w/w).

Local chickpea genotype Uzbekistan-32 was used in this experiment. The planting density was 60 cmx15 cm. Irrigation, pest and weed control were carried out on the conventional management for chickpea. There were two harvests. The first harvest was at flowering stage. One representative plant for each plot was harvested. The parameters of shoot biomass, N content, plant height, the number of pods, the number of nodules and nodule dry weight were analyzed. The second harvest was at the maturity stage. The plants were harvested according to their maturity time, and then were used to estimate the yield based on the plot yield.

Soil chemical and physical analysis

Air-dried samples were analyzed for the total C, N, P, K and soil humus contents. Soil particle distribution was determined using natrium phosphate. The total carbon content, Ctot, was identified by elementary analysis while total nitrogen, Ntot, content was determined by the Kjeldahl method. The molybdenum blue method determined the total phosphorus content, Ptot, in soil. Potassium, K, was determined using the Flame Photometric Method (Riehm, 1985). The Atomic Absorption Spectrophotometer (AAS) was employed to measure calcium chlorite ($CaCl_2$). Soil pH-value was measured by means of electrometer.

Statistical procedures

Data were tested for statistical significance using the analysis of variance package included in Microsoft Excel 97. Comparisons were done using Student's *t*-test. Mean comparisons were conducted using a least significant difference (LSD) test (P=0.05).

Results and Discussion

Chickpea production in Uzbekistan is very low because there are so many factors in soil limiting chickpea growth, such as salinity, drought and appropriate rhizobial strains. Legumes could fix N biologically, but only after they form nodules with *Rhizobium*. The *Rhizobium*-legume symbiosis has received most attention as they are widely deployed in agricultural practices for sustainability of crop yield and recovery of soil fertility (Egamberdieva et al. 2015).

The use of specific microbes which stimulate plant growth allows a considerable decrease in the use of agrochemicals which are now being used for plant growth stimulation. This will positively affect emergence of seedlings and further growth in soils with a poor structure such as those in Uzbekistan. Micro-organisms in the rhizosphere react to the many metabolites released by plant roots. Their interactions with roots help in nutrient uptake of plants, also the adaptation of plants to adverse soil chemical conditions and susceptibility to disease (Bouhmouch, et al. 2005). Soil beneficial microorganisms have been studied intensively because of their potential impact on agricultural productivity (Davronova, 2013).

The effective indigenous rhizobial strains isolated in this study from chickpeas on middle salinated soils of Uzbekistan have the characters of broad host range, high nodulation efficiency, efficient N fixation, great salt tolerance. Selected strains were able to increase chickpea yield and to reduce the percentage of diseased plants caused by *Fusarium* pathogens in salinated soil.

Forty bacterial strains of *Rhizobium* sp. previously isolated from chickpea root were screened for their salt tolerance abilities. Most of strains where able to growth under 3% NaCl, and only 10 strains were able to tolerate up to 5% NaCl. The results showed that the seed dormancy enforced by salinity (5% NaCl) was substantially alleviated and the germination was promoted by selected rhizobial strains from 54 up to 90% (data not shown). Those 10 strains where taken for further studies on their effect on growth and symbiotic performance of chickpea under salt stress condition. The results of study showed that salt tolerant rhizobial strains stimulated root, shoot growth and nodulation of chickpea affected by salt stress (Table 1).

Table 1. The effect of *Rhizobium* sp. strains on the root, shoot growth and nodule number of chickpea grown under saline soil condition

Bacterial strains	Nodules number	Shoot length (cm)	Root length (cm)	Shoot dry mass (g)	Root dry mass (g)
Control	0	16.7	12.4	0.213	0.081
R 1	12	18.9	14.5	0.224	0.095
R 2	11	19.2	14.2	0.236	0.093
R 3	11	18.6	13.9	0.223	0.092
R 4	20	26.1*	19.0*	0.286*	0.142*
R 5	12	19.5	14.9	0.233	0.094
R 6	15	22.3*	15.7*	0.293*	0.116*
R 7	13	19.3	14.9	0.236	0.095
R 8	10	18.2	13.9	0.217	0.089
R 9	16	21.8*	15.4*	0.294*	0.115*
R 10	14	19.9	15.3	0.234	0.097

* Significantly different from untreated control plants at P<0.05

Selected salt tolerant rhizobia had good symbiotic association with chickpea variety Uzbekistan, which previously selected as salt tolerant cultivar (Egamberdieva et al. 2014).

Without inoculation, no nodules were formed in the tested local chickpea genotype Uzbekistan-32; with inoculation, the nodulation rates in all were 100%. The rhizobial strains R4, R6 and R9 alleviated quite successfully the reductive effect of salt stress on percentage of germination (up to 70%) and seedling growth. Inoculation with rhizobial inoculants not only made many nodules formed, but also increased chickpea shoot and root biomass. The shoot length increased by 38%, root length by 31%, shoot dry weight by 27%, and root dry weight by 42% after inoculation with bacterial strain R4. Similar results were revealed almost all bacterial strains. More considerable result were found when inoculation of chickpea with *Rhizobium* strains R6 and R9, which significantly increased shoot and root dry matter by 37-38% and 43-42% above the uninoculated plants, respectively. Inoculation significantly increased the pod number and pod weight compared to control plant (Table 2). According Kyei-Boahen et al. (2002) and Berger et al. (2006) growth potential of chickpea depends on the rhizobia association and plant genotype which together influencing the symbiotic performance.

Bano et al. (2010) reported that bacterial strains adapted to drought stress was effective in the root-nodule symbiosis and also alleviated decreased growth and yield of chickpea imposed by drought stress. The three best, effective strains R4, R6, and R9 showed high stimulatory effect on the root and shoot growth of chickpea seedlings which further were used for field experiments.

The colonization of root associated beneficial microbes in the rhizosphere is important for their beneficial effect on plant growth, especially under stress soil conditions (Zahran, 2011). It has been also observed that the survival of rhizobia in the plant root and soil is affected by nutrient deficiency, salinity, drought and acidity (Slattery et al. 2004). In earlier report Lowendorf (1980) reported that salinity inhibited survival and proliferation of *Rhizobium* spp. in the soil and rhizosphere, and infection process. This study on survival of salt tolerant R4, R6 and R9 strains in the rhizosphere of chickpea grown under saline soil condition indicates that screening for salt tolerant rhizobial strains are essential to improve symbiotic performance of chickpea under salt stress condition. They are able to stimulate plant growth, alleviate salt stress and survive in the rhizosphere of plant under extreme soil conditions.

Table 2. The effect selected salt tolerant effective *rhizobium* sp. on the growth and yield of chickpea under field condition

Treatments	Nodule number	Nodule weight, g/plant	Root dry weight, g/plant	Shoot dry weight, g/plant	Yield of chickpea, dt/ha
Uzbekistan-32 control	18±1.4	0.15±0.01	1.35±0.4	17.63±1.8	10.5±1.4
Uzbekistan-32+R4	54±5.3*	0.89±0.14	2.18±0.64	20.43±2.4	14.5±1.6
Uzbekistan-32+R6	42±3.5	0.49±0.04	2.22±0.11	21.43±3.1	13.8±0.9
Uzbekistan-32+R9	69±7.4*	0.43±0.03	1.77±0.09	21.33±3.3	17.8±2.7

* Significantly different from untreated control plants at $P<0.05$

A field experiment was carried out by applying rhizobial inoculants R4, R6 and R9, which showed the highest nitrogenase activity on a typical salinated soil of Uzbekistan. Our results showed legumes inoculated with *Rhizobium* could increase the nodule numbers, improve the activity of *Rhizobium* and other soil microorganisms, enhance N fixation and promote root growth, so as to increase the yield of legume plants. But the inoculation effect on yield increase mainly depends on the competition for nodulation of *Rhizobium* and the nitrogen fixation efficiency. In order to improve the *Rhizobium* application effects, the key measure is to select rhizobial strains with well adaptation to local soils, strong competition for nodulation as well as efficient N fixation.

Although inoculation with *Rhizobium* could significantly promote chickpea growth and increase pod number (Table 2), but not plant height (data not shown). Meanwhile, inoculation with *Rhizobium* could significantly affect reproductive growth of chickpeas.

This might be reasoned that inoculation with *Rhizobium* could improve N nutrition, promote vegetative growth, particularly root growth, as well as benefit root uptake from soil in chickpea. Similar observations reported in other studies where inoculation of chickpea with rhizobia increased plant growth, ground dry matter, number of pods, seed yield, and nitrogen fixation under various climatic conditions (Fatima et al. 2008).

Well-structured and deep penetrating root system of chickpea and legume-*Rhizobium* association caused to improve the soil physical and chemical properties (Table 3 and 4). Soil bulk density decreased in the 0-30 cm layer from 0.02-0.04 g cm^{-3}, increased the number of water stable aggregates from 2.4 to 3.5%, soil permeability from 2.1 to 14.3% and soil organic matter (humus) increased for 0.02-0.03%. Total nitrogen, carbon and phosphorous content in the soil increased for 0.022-0.033, 0.054-0.084 and 0.007-0.015 $g.kg^{-1}$, respectively. The results revealed that inoculation of chickpea seeds with *Rhizobium* strains can considerable improve soil chemical and physical properties compare the control variation trials. These results suggest inoculation of chickpea seed with *Rhizobium* bacteria has the beneficial effects to both crop production and soil fertility.

Table 3. Soil chemical analysis

Treatments	Ct, $g.kg^{-1}$	Nt, $g.kg^{-1}$	Pt, $g.kg^{-1}$
At the beginning of experiment	0.823±0.01	0.056±0.012	0.159±0.01
Uzbekistan-32 control	0.877±0.02	0.078±0.006	0.166±0.02
Uzbekistan-32+R4	0.989±0.03	0.087±0.014	0.174±0.015
Uzbekistan-32+R6	0.984±0.01	0.093±0,009	0.179±0.04
Uzbekistan-32+R9	0.907±0.03	0.089±0.011	0.174±0.012

Table 4. Soil physical analysis

Treatments	Soil Bulk Density ($g.cm^{-3}$)		Humus content, %	
	0-30 cm	30-50 cm	0-30 cm	30-50 cm
At the beginning of experiment	1.544±0.04	1.680±0.05	0.975±0.03	0.842±0.04
Uzbekistan-32 control	1.439±0.02	1.581±0.06	0.995±0.06	0.869±0.05
Uzbekistan-32+R4	1.441±0.03	1.580±0.08	0.995±0.07	0.867±0.07
Uzbekistan-32+R6	1.437±0.05	1.581±0.04	0.999±0.11	0.870±0.06
Uzbekistan-32+R9	1.445±0.07	1.584±0.1	1.005±0.07	0.872±0.08

The total C, N and P concentrations in soil under conventional tillage system depended on chickpea association with rhizobial strains (Table 3). The growing of chickpea genotypes without inoculation with *Rhizobium* had significant effect on soil fertility compare with *Rhizobium* inoculated ones. Inoculation of chickpea genotypes with *Rhizobium* strains have led to increase soil carbon, nitrogen and phosphorous contents. Lower concentration of organic matter, N and P content under chickpea grown without *Rhizobium* can depend on the reduced microbial activity consequently the reduced C input to soil. According Schimmel and Bennett (2004) N mineralization by soil microbes is the key event in the N cycle making mineral N bio-available, whereas plants only uptake mineral N. Changes in N dynamics in soils are closely connected with altering in microbial activities involved in N cycle by biotic and abiotic factors (Aziz et al. 2011). It has been reported that accumulation of N by chickpea and with association *Rhizobium* strains have a substantial effect on the soil organic matter, but also on microbial activities under stressed condition (Kantar et al. 2007). We have observed that soil nutrients were increased under chickpea grown in association with *Rhizobium* strains R4, R6 and R9. It is most probably related to greater release of exudates and availability of N and C substrates, due to legumes extensive rooting system (Egamberdieva et al. 2015), and the availability of mineral nutrients in soil which are of considerable importance to increasing microbial populations (Khaitov and Allanov, 2014). Chickpea had a versatile capacity to produce greater root exudates and enrich the soil with nitrogen through nitrogen-fixing activities (Tripathi et al. 2015).

Results from the field experiment indicated that rhizobial strains isolated by us could infect more chickpea genotypes with effective N fixation and adaptability to salt and drought conditions. It is very meaningful to widely apply the effective rhizobial inoculants in Uzbekistan, in order not only to increase host N nutrition, improve crop production, increase soil N content and fertility, but also effectively reduce applications of chemical fertilizers, particularly N fertilizers, finally aiming for improving the quality of ecological environment, benefit the maintenance of ecological balance.

Conclusion

Most irrigated fields in Uzbekistan have salinated soils. Accelerated secondary salinization of irrigated soil is recognized as the major agricultural problem in Uzbekistan and the substantial areas under crop production in the country are affected by different level of soil salinity.

The abundance of indigenous *Rhizobium* in these areas is very low because of the lack of chickpea cultivation, the high temperature in summer and cold weather in winter seasons. Inoculation with *Rhizobium* is an effective approach to strengthen N fixation, increase N nutrition and promote yield in chickpea. Therefore, inoculation with the effective rhizobial inoculants might be an important approach to improve chickpea production on salinated soils in Uzbekistan. This study revealed that screening for salt tolerant rhizobial strains are essential to improve symbiotic performance of chickpea under salt stress condition as well as improve soil fertility. Application of inoculation techniques with rhizobial inoculants in legume production has great economical, environmental and ecological benefits. Furthermore, the study shows the potential of phytohormone producing strains R4, R6 and R9 as promising candidate for development of biofertilizer along with nodulating strains to get sustainable yield of chickpea with minimum inputs at marginal land.

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Dynamic surface soil components of land and vegetation types in Kebbi State Nigeria

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Abstract

Land and vegetation are important components of soil and provides many benefits to surface soil including protection against erosion, climate change impact and unacceptable degradation of soil particles. Visual Soil Assessment was used as a mechanism to assess and classify the land and vegetation types of some agricultural sites in Kebbi State, Nigeria. The aim was to get better understanding of the environmental soil function for sustainable crop production in dryland and fadama areas of the State. The assessment was able to put together combinations of different vegetation types and land age classes. It is valued that the land age classes possessed the characteristics of Holocene-natural, Holocene-anthropogeomorphic, Holocene-young-natural, young-anthropogeomorphic, very-young anthropogeomorphic and very-young natural. However, the vegetation types could be related to evergreen forest, short medium forest (scattered clustered), dwarf vegetation (scattered isolated), grass vegetation, thick vegetation, stony-grass vegetation (scattered sparse) and short-length vegetation. The assessment provides an improve understanding of the current status of land and vegetation conditions of the study area and suggested regular soil management for sustainable crop production in the State.

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Introduction

Soils vary on how long they can be used in agriculture without decline in quality and functional status before management may be needed. Assessments and classification of surface soil components, including land and vegetation are helpful in understanding the current status and condition of soil for proper and sustainable soil management in crop production (Usman, 2007). Unfortunately, scientific knowledge of surface soil components and in particular the aspect of assessment and classification of land and vegetation is very limited in Kebbi State, Nigeria (KARDA, 1997). Consistently, the need for background information outlining the major components of surface soil environment in poor research areas is also essential (McFadden and Kneupfer, 1990; Levine, 2001; Lemke et al., 2003; Furian et al., 2011). In the present study, the major components of the surface soil (land and vegetation types) according to the geo-physical context of the current status of the soil environment in Kebbi State are taken into consideration based on visual assessment. This assessment was regarded as a key element for obtaining soil data information, and especially when the methodology and procedure are developed under the general principles on which the

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assessment and classification of soils should be made in the field (IUSS Working Group WRB, 2006). This assessment is highly cost and time consuming, and needs to be carried out according to the purpose intended (FAO, 2006). Soil Survey Staff (1999) noted that visual assessment requires many assessments and classifications that can be related to the surface soil and that facilitate comparisons of both similarities and differences among the soil characteristics for a great variety of uses. In this study, visual assessment was intended to assess and classify the land and vegetation types from 2010 to 2011 in Kebbi State. Therefore, the objective of this study was assessment and classification of land and vegetation types in dryland and fadamasites of Kebbi State Nigeria. This is hoping to ensure better understanding of environmental soil function for sustainable crop production in the State.

Material and Methods

Study area

The study was carried out in Kebbi State Nigeria around Arewa, Argungu, Augie, Birnin-Kebbi and Dandi. These areas were dominated by the tribes of Hausa and nomadic Fulani ethnic groups, whose sources of income depend greatly on farming systems (Usman, 2007). The zone has tropical weather conditions with three seasons: rainy, dry and hot. The annual rainfall is variable and declining, being 600 mm to 875 mm and on average 650 mm during the period 1997 to 2014 (Local Meteorological Record). The monthly temperature in the region ranges from 25°C to 45°C. The State possessed two important agricultural lands namely: dryland (arid – prolong dryness) and fadama (floodplains – significant alluvial clay particles). These two lands remained the key source of income to millions of people in the State. Figure 1 shows the study area and its geographical position in sub-Saharan Africa.

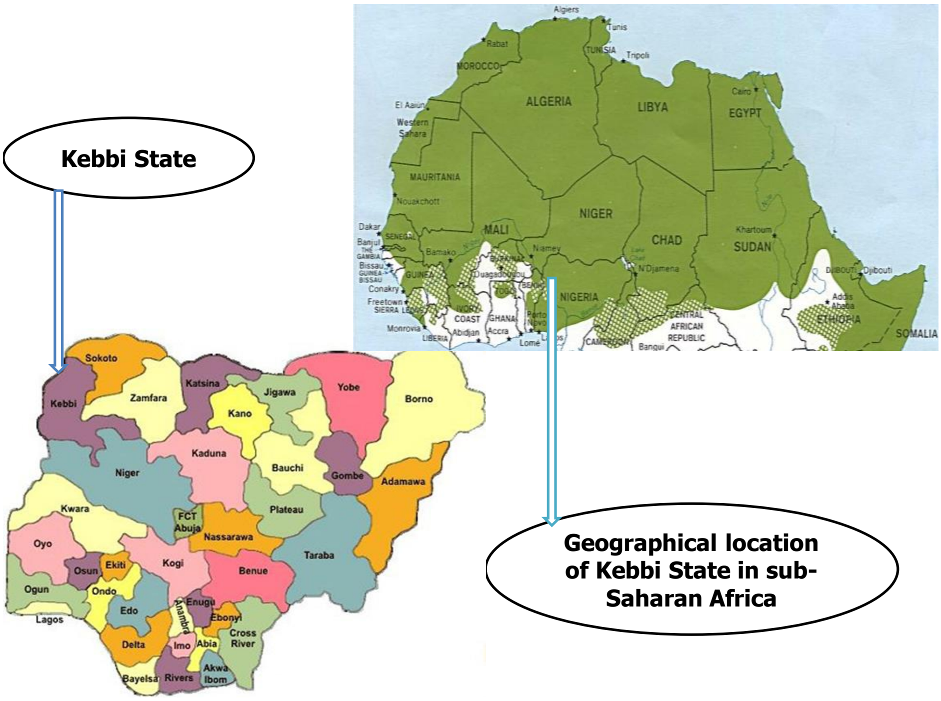

Figure 1. Map of Kebbi State located in Nigeria from sub-Saharan African: Adapted and modified after Perry-Castañeda Library Map Collection (2011)

Visual soil assessment

The assessment and classification were basically carried out according to the general background of Visual Soil Assessment (VSA) in the field from 2010 to 2011. The European Commission (EU, 2010) defined VSA as a direct evaluation of those soil properties, which are visible by the naked eye and which can be evaluated directly in the field. The method was found profitable in assessing the key soil 'states', which are dynamic indicators capable of changing under different management regimes and land-use pressure (FAO, 2008). The VSA has included the description of surface soil characteristics, classification of surface soil components according to a standard system of classification (Soil Survey Staff, 2010). Notably, the field exercise in the study area was divided into two namely – assessment and classification of land age classes and vegetation types.

Classification of land age

Surface land ages were classified according to soil maturity classes. The guidelines provided by FAO (2006) are used to classify all land age in the field. This assessment of land age classes was carefully made at different location of dryland and fadama areas of Kebbi State by adapting the procedure of soil pedon – a smallest volume of soil that can represent a given soil site (Soil Survey Staff, 1999). However, on each site a small surface area 2 m^2 was secured after a preliminary measurement with a tape (Figure 2). The importance of this measurement is only related to pedon as the targets position of the assessment around each study site.

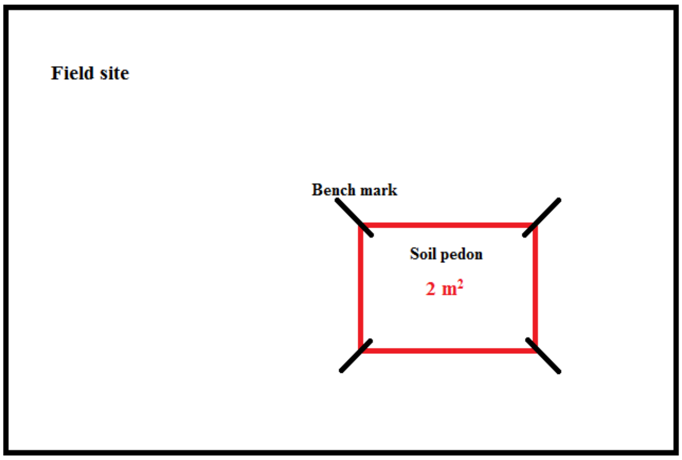

Figure 2. Typical example of schematic diagram of pedon area on the field site

Overall, the VSA and classification of land age classes were made according to the following notation of use extracted from FAO (2006):

a. *Very young natural*: is attributed to surface soil area loss/affected by erosion or deposition of materials such as on tidal flats, of coastal dunes, in river valleys, landslides or desert areas (field observation).
b. *Very young anthropogeomorphic*: this refers to areas with complete disturbance of natural surfaces (and soils) such as in urban, industrial and mining areas with very early soil development from fresh natural or mixed materials (field observation).
c. *Young natural*: this refers to areas affected by erosion or deposition of materials such as sand dunes in desert areas or poor vegetation areas in dryland (field observation).
d. *Young anthropogeomorphic*: this is refers to areas with complete disturbance of any natural surfaces soils such as areas affected by deforestation with early soil development from fresh natural or a mixture of materials (field observation).
e. *Holocene natural*: this refers to areas affected by erosion or deposition of materials in very poor vegetation sites of desert areas leading to gullies or landslides (field observation).
f. *Holocene anthropogeomorphic*: refers to areas affected by human-made relief modifications, such as terracing of forming hills or restriction of flooding by dykes, or surface intensifying or surface contours (field observation).

Classification of vegetation type

Direct observation according to VSA method was used to identify the vegetation cover of the study area. An observation refers only to sites visited in the field and was used to record the major types of vegetation features in the study area. Following this observation, the vegetations of the study areas were assessed and classified according to notation of use outlined in FAO-SWALIM (2007). The summary of the definitions of the key term is given below:

a. *Evergreen vegetation*: Woody perennial plants with a single, well-defined stem carrying a more or less defined crown and at least greater than 5 m tall (measured in the field).
b. *Short medium shrubs:* Woody perennial plants with persistent woody stems without any defined main stem, less than 5 m high (measured in the field).
c. *Dwarf shrubs:* Woody perennial plants with persistent woody stems but are dwarf in nature less than 3 m (measured in the field).

d. *Grasses vegetation:* Short plants characterised by long thin/fine leaves growing wild in bush areas, vary in sizes and shapes (field observation).
e. *Thick vegetation cover:* Woody perennial plants or grasses or shrubs covered most of surface area under survey (based on physical observation).
f. *Short-length plants:* Shrubs or grasses with varying sizes (short and long) less than 3 m and greater than 3 m (measured in the field).

Photographs and images

Photographs and images of some assessment sites were taken. These images were used to show the examples of the land age classes and vegetation types of the study area in Kebbi State.

Results and Discussion

Surface land age classes

The results of land age classes in the study sites are presented in Table 1. The sites are characterised by six different land age classes in dryland and fadama areas. These land age classes are: Holocene natural, Holocene anthropogeomorphic, Holocene young natural, young anthropogeomorphic, very-young anthropogeomorphic and very-young natural (Table 1).

Table 1. Major land age classes around Arewa, Argungu, Augie, BirninKebbi and Dandi

Location	Land age class: FAO (2006)	Agricultural site
Arewa	Young anthropogeomorphic	Dryland
	Holocene natural	Dryland
	Holocene young natural	Dryland
	Very young natural	Dryland
Argungu	Very young anthropogeomorphic	Fadama
	Holocene anthropogeomorphic	Fadama
	Young anthropogeomorphic	Dryland
	Holocene young natural	Dryland
	Very young natural	Dryland
Augie	Very young anthropogeomorphic	Fadama
	Young anthropogeomorphic	Dryland
	Holocene young natural	Dryland
	Very young natural	Dryland
BirninKebbi	Very young anthropogeomorphic	Fadama
	Holocene anthropogeomorphic	Fadama
	Young anthropogeomorphic	Dryland
	Very young natural	Dryland
Dandi	Young anthropogeomorphic	Dryland
	Holocene young natural	Dryland
	Very young natural	Dryland

Field Survey: S. Usman: 2010 – 2011

An observation made, noted that the holocene-natural and holocene-anthropogeomorphic are physically referred to surface soils affected by erosion and human made relief modification, respectively. Also, the young natural, young anthropogeomorphic, very-young anthropogeomorphic and holocene very-young natural are characterised by deposition of new soil particles plus organic materials of plant and animal kinds. Both the holocene-natural and holocene-anthropogeomorphic have similar surface soil nature that is physically undisturbed compared to the surfaces of young and very young anthropogeo-morphic soils, which are physical disturbed. Bodily, the parent materials developed in the surfaces of young and very young anthropogeo-morphic soils are fine, mixed-up and combined with different organic materials. These different materials are examined according to the physical condition of the surface soil appearances of the study sites. The development and formation of surface land cover as well as natural and synthetic changes are considered important factors, which might have contributed to the formation of young and very young anthropogeo-morphic classes (e.g. Colhoun and van de Geer, 1986). Ideally, addition of organic materials changes surface soil appearances by exchanging contact with natural surface soil particles under addition of soil formation process (Harris and Yusuf, 2001; Ghosh et al., 2010). This might have taken part in the transformation and genesis of some surface soil sites characterised as young anthropogeo-morphic condition in the study area (e.g. Figure 3).

Figure 3: Examples of land age classes in the study sites: (a) very young anthropogeomorphic, (b) young anthropogeomorphic, (c) Holocene anthropogeomorphic, (d) very young natural, (e) Holocene natural and (f) Holocene young natural. Photos by Suleiman Usman

Generally, the six land age classes are characterised by the presence of consolidated and unconsolidated sedimentary parent materials, which might have formed from the weathering of rocks 100 to 10,000 years old (FAO, 2006; Morrocco et al., 2007). The results of agricultural activities and other human impact on surface soil, land and natural vegetation cover in most part of the study sites, have also caused tremendous changes in holocene-natural and holocene-anthropogeomorphic classes (Figure 3). Results of such changes have been the formation of new surface lands from young to very-young anthropogeomorphic 10 to 100 years old (Figure 3). Lal (1997) described the conditions of these surface soils as soils with inability to: (a) sustain biomass production and biodiversity; (b) regulate water and air quality by filtering: retain water after passing through, buffering: protect from damaging impact and geochemical cycle); (c) preserve archaeological, geological records; and (d) support socio-economic structure, cultural and aesthetic values (study of art, building) and provide engineering foundation.

Reasonably, if there really is erosion combined with humans' inappropriate activities to which holocene-natural and very-young-natural surface soils were formed in the study sites, they would have undoubtedly caused deterioration of natural surface soil quality of the study sites, as similarly noted by Zhang et al. (2006) in China and Usman (2007) in northern Nigeria. On the contrary, factors such as poor vegetation cover, poor soil management, lack of awareness and poor environmental government policy might have also taken a major part in the occurrence of holocene-natural and very-young natural in the study sites (e.g. Usman, 2007). The formation of many young soil particles would have increased due to combined effect of the stated factors, and this has been a case report in most of Saharan and arid regions of Africa (Colhoun and van de Geer, 1986; McIntosh et al., 2004; Furian et al., 2011).

Vegetation types

The results of vegetation types in the study sites are also presented in Table 2.

Table 2.Different types of vegetation around Arewa, Argungu, Augie, B/Kebbi and Dandi

	Type of vegetation[1]	Study area	Physical importance to surface soil
a	Evergreen vegetation	Argungu	Good for soil and environmental protection.
b	Short medium shrubs (Scattered clustered)	B/Kebbi	Protect soil against devastating wind.
c	Dwarf vegetation (Scattered isolated)	Arewa	Reserve soil moisture, improve soil fertility.
d	Grass vegetation	Argungu	Serve as soil carpeting, reserve soil moisture.
e	Dense (thick) vegetation	Augie	Good for soil and environmental protection.
f	Thorny-grass vegetation (Scattered sparse)	Arewa	Support soil particles by reducing the direct and indirect impact of terrible rains.
g	Short-length vegetation	Dandi	Protect soil against wind and mass movement.

Field Survey: Suleiman Usman: 2010 – 2011, FAO-SWALIM guide (2007)

The result shows that the study sites consist of different vegetation types from BirninKebbi to Argungu through Augie and Arewa to Dandi areas (Table 2). In the Hausa language, most of these vegetation areas are called Kuya (mixed-forest), Kurmin-daji (clustered-forest) and Kali (orchard-forest). Physically, these areas are characterised by fewer annual grasses, thorny tree species and trees of different shapes, length and sizes. Areas dominated by taller trees/shrubs around Argungu, are named as evergreen vegetation and areas covered by shorter and very-shorter flora are classified as dwarf and short-medium vegetation types in Arewa and BirninKebbi, respectively. However, areas covered by thicker or chunky areas (bushy) are classified as dense and short-length shrubs in Augie and Dandi, respectively. Surfaces characterised by present of flora grasses with thorny or prickly plants are classified as 'thorny-grass vegetation' in Arewa. Short medium shrubs and dwarf vegetations are classified according to the condition of plants grown in their respective sites. The physical importance of these vegetation types to surface soil was also assessed based on VSA in the field at that time of assessment. Physically, most of the vegetation plays an important role in binding soil particles, improving soil fertility and protecting surface soil condition against erosion, hence improving the potential of crop productions, sustainability.

The survey also noted that, physically during the rainy season these vegetation types grow productively, and they dried out in dry period. The major plant species observed during the survey are listed in Table 3. The typical examples of the plant species and vegetation types are also shown in Figures 4 and 5, respectively.

Figure 4. Example of some flora in the study sites: (a) *Ziziphusspp (*Magarya), (b) *Adansoniadigitata* (Kuka), (c) *Acacia nilotica*(Bagaruwa) (d) *Hyphaenethebaica* (Goriba) (e)*Piliostigmareticulatum* (Kalgo) and (f)*Azadirachtaindica*(Darbejiya). Photos by Suleiman Usman

Table 3. Major plant species identified in dryland and fadama areas of the study sites

Scientific name[1]	Local name[2]	Land site
Acacia nilotica	Bagaruwar- bushe	Fadama
Acacia nilotica	Bagaruwa	Dryland and Fadama
Acacia senegalensis	Dishe	Fadama
Adansoniadigitata	Kuka	Dryland and Fadama
Anogessusleiocarpus	Marke	Fadama
Annona senegalensis	Gwanda-daji	Dryland
Azadirachtaindica	Darbejiya	Dryland and Fadama
Balanitesaegyptiaca	Aduwa	Dryland and Fadama
Detariummicrocarpum	Taura	Dryland and Fadama
Eucalyptus spp	Turare	Dryland and Fadama
Faidherbiaalbida	Gawo	Dryland and Fadama
Ficus ovata	Gamji	Fadama
Ficuspolita	Durumi	Fadama
Ficusthonningii	Chediya	Dryland and Fadama
Gmelinaarborea	Sabara	Dryland and Fadama
Hyphaenethebaica	Goriba	Dryland and Fadama
Khayasenegalensis	Madachi	Fadama
Moringaoleifera	Zogala	Dryland and Fadama
Parkiabigbosa	Dorawa	Dryland
Piliostigmareticulatum	Kalgo	Dryland and Fadama
Psidiumguajava	Gwaba	Dryland
Rogeriaadenopylla	Loda	Dryland and Fadama
Tamarindusindica	Tsamiya	Fadama
Vernonaamygdalina	Shiwaka	Fadama
Vitellariaparadoxa	Kadayi	Dryland and Fadama
Vitexdoniana	Dinya	Fadama
Ziziphusspp	Magarya	Fadama

[1]Field Survey: Suleiman Usman: 2010 – 2011, [1]PROTA Precursor (2002); [2]Hausa language

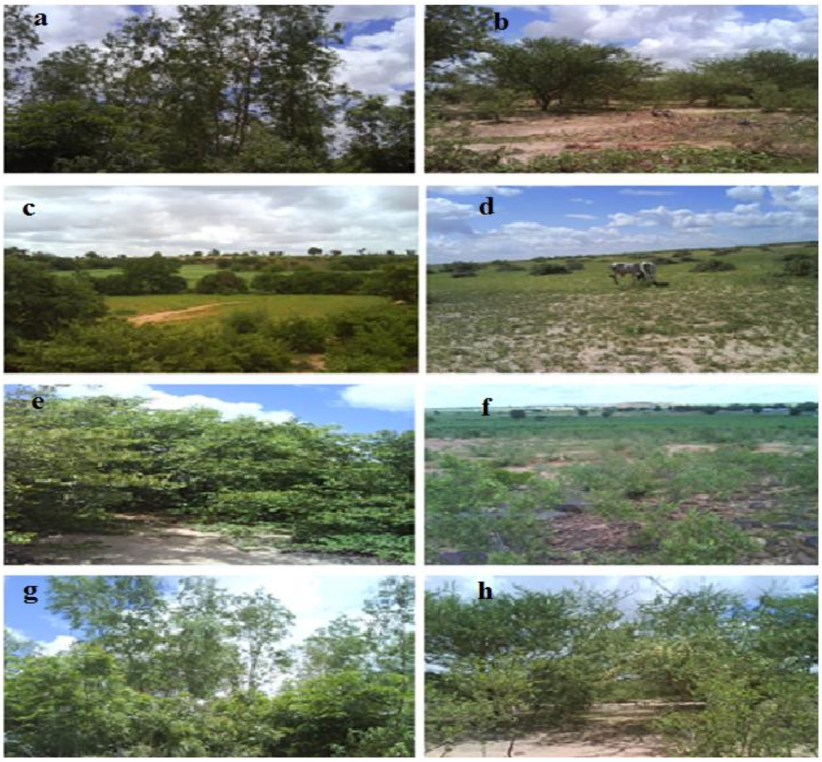

Figure 5. Examples of major vegetation types in the study sites: (a) evergreen, (b) short medium, (c) dwarf, (d) grazing, (e) dense (thick) tree shrubs, (f) stony-grass-shrubs, (g) short-length, (h) medium thorny-tree shrubs. Photos by Suleiman Usman

Conclusion

In a framework of précised VSA carried out between 2009 and 2010 in Kebbi State, the evaluation was able to put together combinations of different environmental soil components mainly vegetation types and land age classes. The information obtained can be considered as pre-requisites for the attainment of the basic requirement and objective of environmental aspect of surface soil management for achievable sustainable crop production in the study sites. The study provides an improve understanding of the current status of surface soil environment in term of land age and vegetation types of the study sites. It is therefore, suggest that a regular observation of the environmental components of soil would provides more detail information on land and vegetation conditions for proper and permanent sustainable crop production in the entire Kebbi State agricultural systems.

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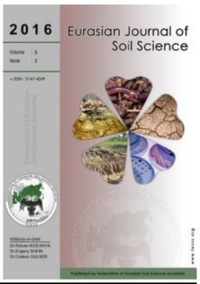

An experimental investigation of rill erosion processes in lateritic upland region: A pilot study

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Abstract

The present paper is based on field investigation and measurement of rill erosion processes at Rangamati Experimental Station (Medinipur, West Bengal in India). In rill experiments, three different natural rills were studies in field for understanding of the dynamics of soil erosion processes of a rill catchment area. Geometric and morphological characteristics of each rill catchment area were analyzed. Results showed the widening, deepening and extended of rills because of sidewall sloughing, knick-points and head-ward erosion during surface runoff process. Progressive increases of rill volume were observed in the upper, middle and lower catchment with the change of time. Rill area has increased by runoff processes of 4.2 % and 6.8 % for Rill-A and Rill-B respectively. These processes are depends on surface coverage, soil texture, slope gradients and runoff velocity.

Keywords: Rills, rill geometry, rill morphology, rill erosion process

Introduction

Rill-gully erosion is major land degradation processes, causing both impacts on-site and off-site soil loss through sediment deposition in downstream environments. Rills were generated by water erosion and consist of several characteristics including a steep incised channel with an active headcut (Bruno et al. 2008; Stefano et al. 2013; Shit et al., 2014). The expansion of rills is due to the multifarious interface of soil properties with a high spatial and temporal variability (Nachtergaele et al., 2001), in which the morphology of a rill and the rill's headcut morphology may be determinant over and above stochastically motivated processes (Sidorchuk, 2005; Flores-Cervantes et al., 2006; Shit et al., 2013). This leads to great difficulties in quantifying soil erosion processes and makes soil erosion measurements hardly comparable (Knapen et al., 2007; Auerswald et al., 2009).

The mechanisms of inter-rill and rill erosion development by flowing water are completely different. The detachment in inter-rill erosion is caused and enhanced by drop-impact (Beuselinck et al., 2002) and, in addition to the soil's intrinsic characteristics (Kuhn and Bryan, 2004; Le Bissonnais et al., 2005; Brodie and Rosewell, 2007). Earlier study also stated that rill erosion is caused by the concentrated flow of water (Bryan, 2000; Govers et al., 2007; Knapen et al., 2007) and is considered to be the most important process of sediment production (Cerdan et al., 2002). The resulting rills may be persistent and develop into gullies, hindering further land use (Woodward, 1999; Vandekerckhove et al., 1998). Especially on fallow land and

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shrub land, rills can develop without disturbance by land management measures. In India, huge areas of fallow land and wasteland exist (Pandey et al., 2007). Thus rills can develop very fast and cause high soil losses and about 5334 m-tonnes of soil are being removed annually due to various reasons (Pandey et al., 2007).

Most experimental work about rill erosion has been carried out in the laboratory (Brunton and Bryan, 2000; Mancilla et al., 2005) and under the field conditions with different textures and natural or simulated rainfall (De Santisteban et al., 2005; Rejman and Brodowski, 2005). The main problem the laboratory experiments is that the results cannot be easily transferred to natural rills (Wirtz et al., 2010, 2012). In field condition rill erosion measurements are still lacking and there is a recognized need to perform field experiments to ascertain the role of rills in soil erosion. As the observation of erosion in the field is subordinated to the stochastic character of the erosion events and to a high dependency of the measurement technique standardized and reproducible field experiments are needed. Therefore, the present study focused on the mechanism of rill erosion process and quantifies the rill erosion at Rangamati experimental station, Medinipur (West Bengal, India).

Material and Methods

Study area

Experimental work has been conducted at Rangamati in Paschim Mednipur district (West Bengal, India), extended between 22°24' N latitude and 87°17' E longitude (Figure 1). The parent material of the study area consists of tertiary and secondary lateritic, sands, silts and clay. Mean annual rainfall is about 1450 mm, distributed in 110 rainy days while mean annual temperature is 28 °C and mean monthly temperatures range between 8°C in January and 43 °C in June (Shit et al., 2013). The area is dominated by low shrubs, *Eragrostis cynosuroides* grass and some wild species. The land cover at south-side of Rangamti is covered with abandoned fields and extensively grazed by cattle's. Cossi River side characterized by agricultural land use and is comprised mainly of crop-farming and vegetables (Shit et al., 2014). The landscape is characterized by hard rock up-lands, barren lateritic covered area and non-arable lands. The main erosive processes that affect the landscape are related to runoff waters and mass failures that causes gully erosion.

Figure 1. Location of the study areas (Rill –A, Rill –B, Rill –C)

Description of Rill sites

The study was carried out into rill channel data at three different sites in Rangamati badland at Midnapur town (West Bengal). The texture of soil was characterized based on the FAO (2006) guidelines. Rill-A (Figure 1 A) was situated on the steepest slope (average 5º) with a catchment area of 59.04 m2. The total rill length was 17 m with a maximum width of 1.21 m and 0.316 m depth (Table 1). Vegetation and rock fragments cover approximately 7% of the rill area. Three cross-sections were drawn, such as 0.0275 m^2 at 5 meters; 0.1047 m^2 at 11 meters and 0.1154 m^2 at 17 m of the tested gully length. The soil texture was classified as medium clay silt with a particle 2.70 gcm^{-3} and moderate bulk density of 1.61 g cm^{-3}.

Table 1. Rainfall data for experimental events

Date	Rainfall (mm)	Total duration (in hours)	Intensity of rainfall (mm/h)
08.09.2014	14.5	1.30	9.66
16.09.2014	17.0	1.0	17.00
21.09.2014	20.0	1.40	11.97
27.09.2014	24.5	2.20	10.51

Rill-B was characterized by an average slope of 4º and with a catchment area of about 160 m^2 (Figure 1B). The maximum width was recorded as 1.40 meter and the depth of 0.35 meter. Only 5% of the rill was covered by rock fragments and vegetation cover. The cross sections were measured in three areas as follows: 0.1317 m^2 at 25 m; 0.133 m^2 at 42 m and 0.0268 m^2 at 52 m (Table 1). The soil texture is classified as silt clay loam with a low bulk density of 1.3 gcm^{-3}. The particles density value reaches to 2.56 gcm^{-3}.

Rill -C developed in a loam soil with a high content of sand (24 % of the find soil) and coarse fragments (11% of the total soil). An average slope of the rill area was recorded as 7˚ and catchment area of about 136 m^2 (Figure 1C). The maximum width of the rill was estimated 1.21 meter and maximum depth of 0.31 meter. Vegetation and rock fragment cover about 15% of the entire rill area. The cross sections were measured at 11 meter, 24 meter and at 34 meter with the corresponding areas of 0.1850 m^2, 0.1026 m^2 and 0.1003 m^2 respectively. The bulk density was recorded as 1.41 gcm^{-3}, moderate and particles density was 2.58 gm^{-3}.

Monitoring rill morphology

The morphological characteristics of the rill areas were measured at 0.5 m interval. The information is recorded during the period between 06th and 29th September, 2014. The rainfall was recorded self recoding raingague in field (Table 1). Runoff was reassured using dry tracer techniques (Shit and Maiti, 2012). The transport sediment was measured within the rill at particular rill end by sediment concentration in the samples. Rills cross section was measured at each measuring point with thin metal sticks. The distance between ground level and rill bottom was measured in before and after rainfall for estimate the widening rate of rill erosion and also photographs captured during the experiment and channel widening was determined from capture photos at particular rainfall events.

Rill erosion measurement

The volume and surface area of the entire gully system was estimated through measurements of width, depth, and length of cross-sectional and length profiles using a 30 meter long tape. We identified three gully cross-sections based on the homogeneity of the gully profile. For each gully, cross section widths and depths were measured along the gully channels. In order to quantify gully volumes and gully cross-sectional morphology, 204 cross-sections were quantified with an equal number of gully segments. The maximum depth (D, in meters), top width (TW, in meters) and bottom width (BW, in meters) of the bankfull channels were estimated. The cross sections were surveyed by a rillmeter (Bruno et al., 2008). For each section, width (w) at the top of the cross section, maximum scour depth (H), cross section (A) and wetted perimeter (C) were measured (Figure 2 and 3). The surface area of entire gully system digitized and cross-checked with the surface area estimated with physical measurements of gully cross-section segments.

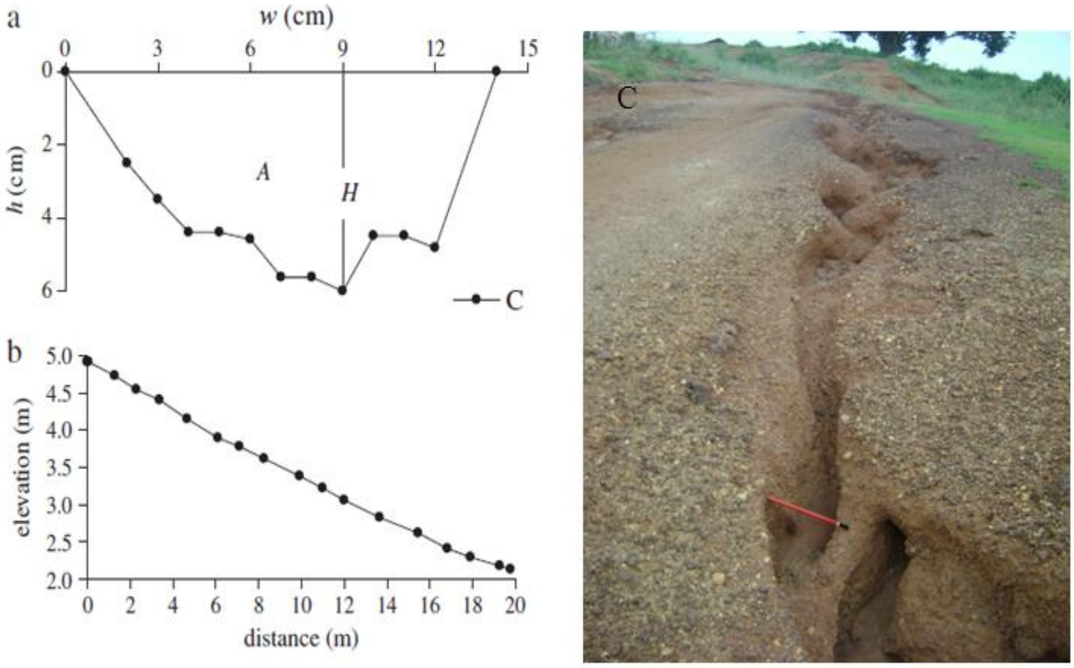

Figure 2. Example of the relief of (a) a rill cross section and (b) rill profile, (c) View of the Rill-C surveyed at Rangamati, Medinipur (after Stefano et al., 2013)

Figure 3. Rill erosion measurement

Once the rill size was determined from the cross-sectional measurements, the rates of erosion were calculated by determining the change in dimension (width, depth, and length) of the different gully segments. The eroded volume V (m^3) of each gully segment was calculated using the cross-sectional dimensions and distance between cross-sections (Eq. 1).

$$V = \sum_{i=1}^{n} L_i A_i \quad \text{.......................................} \quad \textit{Eq. 1}$$

Where, L_i is the length of considered rill segments (m) and A_i is the representative cross-sectional area of the rill segments (m^2).

Results

Rill bed morphology

The longitudinal elevation profiles of the rill shows a succession of steps and pools (Figure 4). Two well defined pools were formed. The mean pool depth was 0.012 meter and the average value of pool length was 0.023 meter. The estimated pools were not symmetric and the downstream well was steeper (7º) than upstream well (13º). Between the depths of 0.026 meter, all pools were separated by steps. The slope gradient of the step surface was very close to the average slope rill width (0.011 to 0.037 meter) (Figure 5).

Figure 4. Rill geometry change during the period between 06th September, 2014 (left) and 29th September, 2014 (right)

Figure 5. Rill erosion during 06.09.2014 (left side) and 29.09.2014 (right side) at Rangamati experimental station (A & B = Rill-B; C & D= Rill-C)

Quantify rill geometry

Rill- A

Geometrical characteristics of the rill-A was assessed in different time periods (Table 2). In preliminary period (Day 1) the average depth was 18.42 cm, whereas the depth was increased up to 19.09 cm five days later. There was significant increase of minimum depth of rill (except in Day 5) in the entire study period (*P<0.01*). Consequently, continuing decrease of maximum depth of rill was observed from Day 2 onwards (*P<0.04*). Results also showed gradual increase of average depth of rill in the study period. The average top width of Rill-A showed steady increased with the time (*P<0.0001*); however, the average bottom width of the Rill-A not varied considerably with the change of time (*P<0.03*). Moreover, ongoing changes of the perimeter of the rill side were observed (*P<0.004*), although no such significant information was derived in gradient in the study site.

Rill-B

Minimum depth of the rill-B was increasing trend from date 1 to 3 and thereafter was a decreasing trend from date 4 to date 5 (Table 3). However, no such significant variation of maximum depth of rill-B was observed during the study period (*P>0.40*). Significant variation of average depth of rill –B was observed (*P<0.008*). Minimum value of top width of rill-B showed increasing trend and it significantly varied in the study area (*P<0.002*). Consequently, average value of top width showed increasing trend (*P<0.04*). Very less variation of average value of bottom width was observed in the study site (Table 3). The average perimeter of rill-B showed regular increasing trend with the change of time (*P<0.001*). However, significant variation of gradient was also observed in rill development (*P<0.03*).

Table 2. Descriptive Statistics of rill geometry: Rill-A

Date	Descriptive Statistics	Depth (cm)	Top width (cm)	Bottom width (cm)	Perimeters (cm)	Gradient (Slope)
Date-1	Minimum	4.40	35.00	16.00	41.00	1.00
	Maximum	40.50	118.00	85.00	131.00	19.00
	Median	19.30	75.00	26.00	94.50	4.00
	Average	18.42	73.79	32.97	87.15	6.06
	SD	9.18	25.88	16.70	28.05	4.93
	CV (%)	49.84	34.60	50.64	32.19	81.36
	Skewness	0.29	0.06	1.31	-0.32	1.61
	Kurtosis	-0.44	-1.18	1.46	-1.14	2.84
Date-2	Minimum	4.60	35.20	16.10	31.00	2.00
	Maximum	41.60	120.10	85.10	120.20	20.00
	Median	19.55	70.15	26.25	94.60	5.00
	Average	18.56	74.92	33.19	87.37	6.24
	SD	9.26	26.17	16.66	27.95	4.24
	CV (%)	49.87	35.40	50.19	31.99	67.93
	Skewness	0.33	0.15	1.30	-0.31	1.22
	Kurtosis	-0.33	-1.23	1.46	-1.15	1.73
Date-3	Minimum	5.00	35.20	16.20	31.30	2.00
	Maximum	40.80	118.30	85.20	141.30	21.00
	Median	19.75	80.30	26.25	94.65	5.00
	Average	18.70	75.34	33.06	87.69	6.56
	SD	9.16	25.28	16.95	28.08	4.43
	CV (%)	48.97	33.11	51.27	32.13	67.58
	Skewness	0.29	0.05	1.24	-0.29	1.21
	Kurtosis	-0.45	-1.15	1.32	-1.19	1.73
Date-4	Minimum	5.20	35.30	18.30	40.40	3.00
	Maximum	31.50	120.50	85.40	121.50	22.00
	Median	19.90	75.40	26.35	94.85	5.50
	Average	19.05	76.35	33.73	88.08	7.09
	SD	9.04	25.96	16.67	27.91	4.49
	CV (%)	47.46	34.45	49.44	32.05	63.41
	Skewness	0.25	0.05	1.25	-0.37	1.31
	Kurtosis	-0.36	-1.20	1.37	-1.18	2.01
Date -5	Minimum	5.00	35.30	17.40	31.40	3.00
	Maximum	31.60	120.60	85.40	131.60	23.00
	Median	19.95	75.55	26.40	95.00	6.00
	Average	19.09	77.42	33.80	89.82	7.53
	SD	9.02	25.95	16.68	27.88	4.65
	CV (%)	47.26	34.41	49.35	31.75	61.80
	Skewness	0.25	0.05	1.25	-0.29	1.33
	Kurtosis	-0.34	-1.20	1.35	-1.17	2.10

Rill-C

In Rill-C, gradual increase of minimum depth of rill was observed, except in day 3; however, gradual expansion of maximum depth was also observed (except in date 2). Significant variation of average depth of rill was found in the study area (*$P<0.05$*). The average top width of the study area was varied from 70.74 to 76.32 cm and significant increasing trend of top width was also documented (*$P<0.03$*). The maximum bottom width of the study area was varied from 50.10 to 78.60 cm. Significant variation of bottom width of the study area was evidenced with the change of time (*P<0.05*). There was a gradual increase of average perimeter (varied from 82.53 – 88.03 cm) was documented (Table 4); whereas, any significant variations were not found in the study area (*P>0.11*).

Table 3. Descriptive Statistics of rill geometry: Rill-B

Date	Descriptive Statistics	Depth (cm)	Top width (cm)	Bottom width (cm)	Perimeters (cm)	Gradient (Slope)
Date - 1	Minimum	2.00	22.00	7.00	39.00	1.00
	Maximum	35.10	140.00	134.00	146.00	14.00
	Median	16.70	65.00	31.50	81.00	4.00
	Average	16.62	61.85	27.28	73.13	5.13
	SD	8.80	18.84	25.69	18.87	3.21
	CV (%)	52.97	28.61	68.90	23.55	62.68
	Skewness	-0.10	0.51	1.89	0.32	1.06
	Kurtosis	-1.00	2.13	4.42	0.94	0.27
Date - 2	Minimum	2.20	22.40	5.30	40.00	2.00
	Maximum	31.40	120.40	134.40	148.00	17.00
	Median	16.50	60.50	31.00	73.50	4.00
	Average	16.96	62.75	31.74	76.90	5.41
	SD	9.01	21.39	25.97	22.49	3.31
	CV (%)	56.09	34.09	67.05	30.44	61.17
	Skewness	0.07	1.62	1.83	0.71	1.14
	Kurtosis	-0.72	4.38	4.00	3.36	0.63
Date - 3	Minimum	5.70	22.40	5.30	41.00	2.00
	Maximum	35.60	120.50	104.30	148.00	17.00
	Median	16.70	66.00	27.00	82.50	4.00
	Average	16.99	64.44	35.06	79.43	6.05
	SD	8.80	20.09	14.20	22.88	5.45
	CV (%)	52.97	32.70	45.72	28.81	90.08
	Skewness	-0.10	-0.56	1.21	-0.34	3.13
	Kurtosis	-1.00	-0.51	1.61	-0.41	14.11
Date - 4	Minimum	3.60	22.50	5.60	45.00	2.00
	Maximum	30.60	120.80	134.70	149.90	18.00
	Median	18.00	69.00	27.00	84.00	4.00
	Average	17.85	65.32	36.39	81.97	6.05
	SD	9.81	20.13	14.12	21.74	5.45
	CV (%)	54.96	31.29	44.98	26.52	90.08
	Skewness	-0.13	-0.52	1.17	-0.21	3.13
	Kurtosis	-1.21	-0.49	1.58	-0.55	14.11
Date - 5	Minimum	2.10	22.60	5.80	40.50	3.00
	Maximum	35.70	120.90	134.80	149.30	19.00
	Median	18.00	66.00	23.50	87.50	4.00
	Average	17.93	66.41	37.42	86.58	6.05
	SD	9.72	20.41	14.17	25.78	5.45
	CV (%)	54.19	32.18	51.66	32.39	90.08
	Skewness	-0.13	-0.15	1.37	-0.47	3.13
	Kurtosis	-1.19	-0.89	1.28	-0.70	14.11

Rill development processes

Rill -A

The depth and width of the Rill-A valley bottom was estimated during the period between 08th and 27th September 2014. In the upslope, the total volume of the study area was varied between 17.83 and 20.44 m (mean±S.D. 19.25±1.04). In the middle slope and lower slope, the estimated average volume was 87.33m and 73.41m respectively (Table 5). The total length of the rill-A at upper slope was recorded as 5m, whereas, the recorded value in the middle and lower slope was 6 m. Significant changes of rill volume in the middle slope (*P<0.0011*) and down slope (*P<0.007*) were observed.

Table 4. Descriptive Statistics of rill geometry: Rill-C

Date	Descriptive Statistics	Depth (cm)	Top width (cm)	Bottom width (cm)	Perimeters (cm)	Gradient (Slope)
Date - 1	Minimum	3.00	36.00	9.00	45.00	3.00
	Maximum	31.00	108.00	59.00	131.00	14.00
	Median	20.10	72.00	22.00	83.00	7.00
	Average	18.37	70.74	25.03	82.53	7.31
	SD	7.35	20.79	10.79	19.33	5.46
	CV (%)	37.98	28.99	43.11	22.87	74.73
	Skewness	-0.36	0.06	0.66	0.04	4.64
	Kurtosis	-0.46	-0.83	-0.34	-0.18	30.39
Date - 2	Minimum	3.00	39.30	11.20	45.20	1.00
	Maximum	35.20	121.10	78.20	131.30	17.00
	Median	20.00	72.00	22.50	83.00	6.00
	Average	19.41	71.06	25.34	83.93	6.65
	SD	7.26	20.85	10.81	21.29	3.18
	CV (%)	38.32	29.35	42.65	25.67	47.84
	Skewness	-0.38	-0.01	0.63	-0.44	0.67
	Kurtosis	-0.60	-0.71	-0.40	1.23	-0.13
Date - 3	Minimum	2.80	39.40	12.70	63.20	3.00
	Maximum	31.50	121.50	78.30	141.60	17.00
	Median	20.10	72.00	22.00	83.00	7.00
	Average	19.66	71.74	26.03	84.53	7.31
	SD	7.35	20.79	10.79	19.33	5.46
	CV (%)	37.98	28.99	43.11	22.87	74.73
	Skewness	-0.36	0.06	0.66	0.04	4.64
	Kurtosis	-0.46	-0.83	-0.34	-0.18	30.39
Date - 4	Minimum	3.10	39.50	11.40	45.40	3.00
	Maximum	31.80	121.70	78.60	129.50	19.00
	Median	18.00	61.50	21.00	78.00	5.00
	Average	21.28	74.99	25.50	87.68	5.99
	SD	6.18	24.53	27.18	26.00	2.95
	CV (%)	35.76	41.59	55.61	34.81	49.28
	Skewness	-0.12	0.20	1.55	-0.40	0.69
	Kurtosis	-0.30	-0.56	2.20	-0.45	-0.26
Date - 5	Minimum	3.10	36.60	11.80	45.60	4.00
	Maximum	31.70	122.00	50.10	130.10	19.00
	Median	19.60	64.50	24.00	81.50	5.00
	Average	24.08	76.32	27.93	88.03	5.99
	SD	6.39	25.09	14.56	26.14	2.95
	CV (%)	35.33	40.26	52.15	33.94	49.28
	Skewness	-0.18	0.12	1.52	-0.51	0.69
	Kurtosis	-0.46	-0.52	2.26	-0.27	-0.26

Table 5. Geometric characteristics of the surveyed rills

Rills	N	Total length, L (m)	Total volume, V (m)				
Rill – A			**Day-1**	**Day-2**	**Day-3**	**Day-4**	**Day-5**
Upslope	10	5	17.825	18.308	19.372	20.307	20.435
Middle slope	12	6	85.671	86.891	87.273	88.123	88.707
Lower slope	12	6	72.142	72.431	73.439	74.347	74.693
Rill – B							
Upslope	50	25	268.445	271.3698	284.6438	297.2735	325.579
Middle slope	34	17	106.641	107.5913	109.4451	135.7981	183.394
Lower slope	18	9	35.8430	37.3594	38.803	43.04863	43.627
Rill – C							
Upslope	22	11	118.844	120.140	131.197	132.4593	132.361
Middle slope	26	13	137.399	140.3138	137.241	141.666	145.926
Lower slope	20	10	57.4650	61.9208	63.4534	63.75795	60.5008

N= number of surveyed cross sections

Rill –B

In Rill-B, the average volume of upslope rill was documented as 289.46 m and volume of upslope varied between 268.44 and 325.58 m. In the middle slope, volume is varied from 106.64 to 183.39 m (mean±S.D. 128.57±29.48). In the lower slope, the volume is varied from 38.84 to 43.63 m. The length of the rill of the upslope, middle slope and lower slope was recorded as 25m, 17m and 9m respectively (Table 5). Significant changes of rill volume were observed with the change of time in the up slope ($P<0.007$) and lower slope ($P<0.002$).

Rill -C

In rill-C, the average volume of upslope was 127 m with standard deviation of ±6.16. In the middle slope, the volume is varied from 137.40 to 145.93m (mean±S.D. 140.51±3.20). In the lower slope, volume of the rill was varied form 57.45 – 60.51m. The rill length of the middle slope was slightly higher in comparison to up slope and lower slope. However, the results showed an increasing trend of rill volume in the up slope, middle slope and down slope with the change of time (Table 5).

Estimating rill erosion rates

Table 5 represents the erosion rate for the Rill-A, Rill -B, and Rill -C. The increase in the erosion rate of the Rill-C, between 06.09.2014 and 29.09.2014 can be explained by recent widening and deepening of the rill at lower section (Figure 6). The areal extension of the rill was estimated during the period between 06.09.2014 and 29.09.2014 (Figure 6). The erosion rate has increased from 06.09.2014 to 29.09.2014 (11.4 %) in Rill-C area. During study period rill area has increased by 4.2 % and 6.8 % in Rill-A and Rill-B (Figure 6). The rill size from the cross-sectional measurements and mean rill erosion rate has been estimated since the incision period (from 06.09.2014 to 29.09.2014), representing 21.8 cm per year in the contribution small rill watershed. Present analysis also illustrated that the rill erosion rate was accelerated significantly since rainfall period in all the study area of rill development.

Discussion and Conclusion

Rill erosion is the result of the combination of different processes including headcut erosion, sidewall sloughing, tunnelling, micro-piping, slaking piping and sapping (Knapen et al., 2007; Wirtz et al. 2012). Our study showed runoff rates were the prime cause of rill development in the study site because of toppling, knickpoints and subsidence processes effect on rill sidewalls. During the observed runoff events, sidewall sloughing and headcut erosion processes caused 23% and 29% respectively of total rills erosion. However, the successive field studies revealed that channels widening and extended through sidewall sloughing and headward erosion. The valley lengthening is considerable due to high intensity of energy available from intensive rain that acted on steep slope. The rates of increase in length of rills are not same everywhere, which varies according to upslope contributing area, gradient, length, texture, etc. Valley widening is mainly performed by basal erosion along valley sides and the subsequent slab failure. Continuous removal of materials from the base of valley sides is to be maintained for active valley widening. Survey shows that the extent of widening increases manifold after the junction with a tributary or entering a gentler course. The concentrated energy along the channels was responsible for valley deepening.

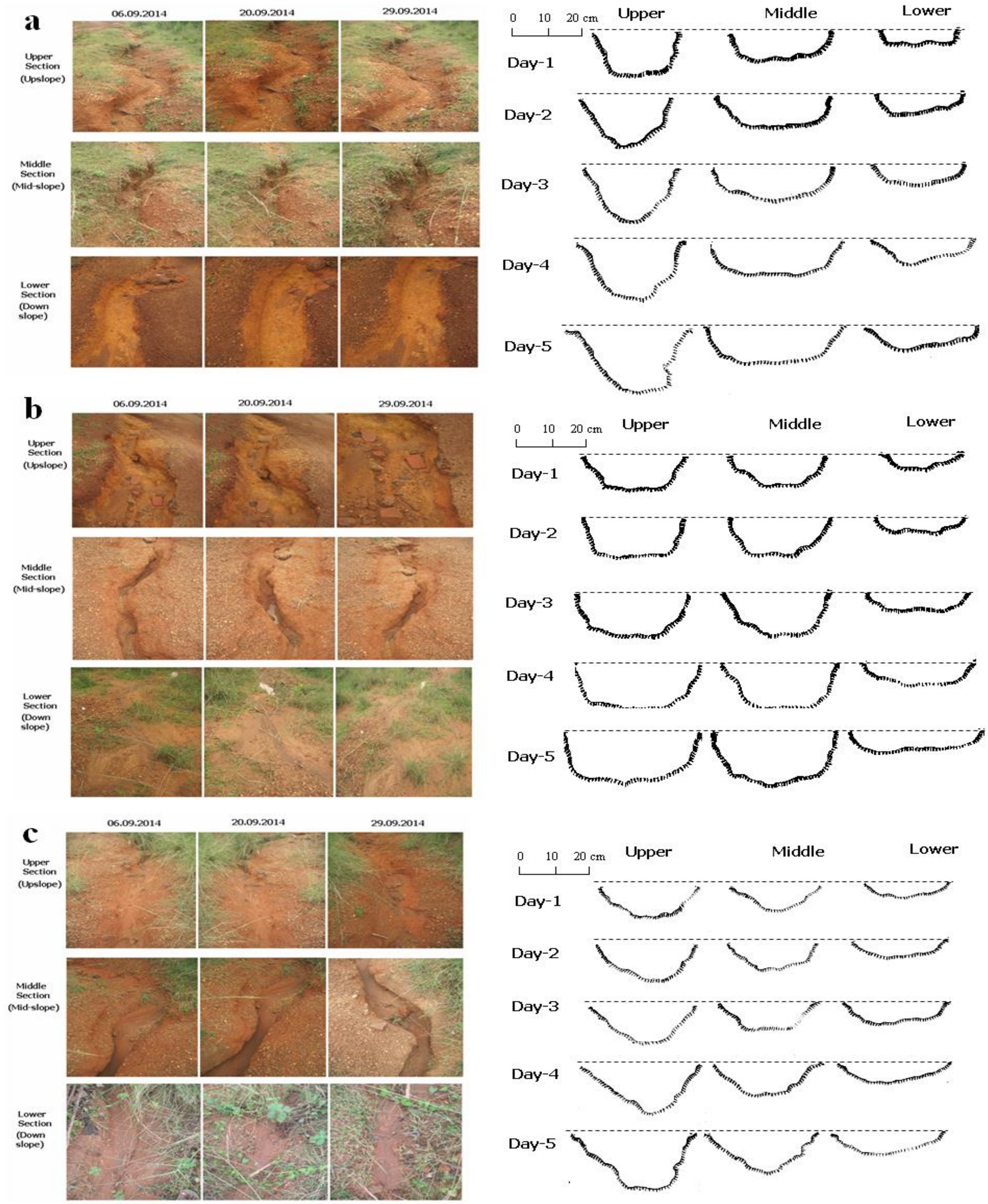

Figure 6. Rill erosion during 06.09.2014 and 29.09.2014 with field photo corresponding cross-sections, (a) Rill-A (b) Rill-B, and (c) Rill-C

The records of the measurements showed that some of the favourable points experienced down-cutting to an amount of 6.0 to 7.0 cm respectively during rainfall event period of 2014. The down-cutting was assisted by the knick development at the source region where surface runoff collected from sheet flow fell from a certain height. This down-cutting and associated back wasting helped in the removal of both lateral and basal support of the materials at the source, and thus an over-hanging slope developed. This slope, thus, retreated by the dislodgement of the overhanging materials which led to valley lengthening. Intense overland flow erosion in the form of rills can wreck barren land and generate environmental tribulations. The present study examined the lateral expansion of channel banks and deepening of channel under the condition of shallow overland flow. However, further experiment is needed at large scale to assess the rill development for its management practices in the study site.

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Influence of composted tobacco waste and farmyard manure applications on the yield and nutrient composition of lettuce (*Lactuca sativa* L. var. capitata)

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Abstract

The use of organic wastes in agriculture, forestry and land reclamation has been increasingly identified as an important issue for soil fertility, soil conservation and residue disposal. Using organic wastes in agriculture helps not only to dispose these materials economically, but also reduces negative effects on the environment. In the present study, composted tobacco waste (CTW) combined with farmyard manure (FM) at different ratios was applied to Typic Xerofluvent soil, and the influence of these amendments on the yield and nutrient composition of butter head letttuce (*Lactuca sativa* L. var. capitata) were investigated. The experiment was conducted in 18 parcels in a randomized-block design with three replications at the Agriculture Faculty's Research Farm of Ege University in Menemen plain, in the Western Anatolia Region of Turkey (38°58′35.51″-38°58′36.03″N; 27°03′84.56″-27°03′89.81″E). Organic materials were applied to the soil after composting. The treatments were (1) control, (2) 12.5 t ha^{-1} FM + 37.5 t ha^{-1} CTW, (3) 25 t ha^{-1} FM + 25 t ha^{-1} CTW, (4) 50 t ha^{-1} FM, (5) 50 t ha^{-1} CTW, and (6) 37.5 t ha^{-1} FM + 12.5 t ha^{-1} CTW. The maximum yield was obtained during the 1st vegetation period (62,7 t ha^{-1}) in the 100 % CTW application. On account of the 2nd vegetation period's coinciding with winter and the coldness of the months December, January and February, there happened a slowdown in the lettuce yield. The highest total yield of lettuce in both vegetation periods (102.7 t ha^{-1}) was determined in 100% CTW application parcels. The lower lettuce yields were determined in the control parcels. CTW and FM applications raised N, P, K Ca, Mg, Na, Fe, Zn and Mn contents of the lettuce. According to the results obtained, it can be said that CTW can be used in agricultural fields just like FM.

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Introduction

Increase of population and the industrial development produced an enormous amount of organic residues that generate great environmental problems nowadays. Potential solution is better use of organic residues resulting from human activities, such as sewage sludge, manure and mankind own organic residues, such as tobacco waste. The appropriate agricultural use of organic wastes can become advantageous for the humankind, because it allows recycling, lessening the pollution problems, as well as the improvement of the physical conditions, chemistries and biotic of the soils (Brito et al., 2007). Benefits of organic wastes and

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compost amendments to soil have been reported by many researchers (Roig et al., 1987; Doran and Parkin, 1994; Darwish et al., 1995; Drinkwater et al., 1995; Stamatiadis et al., 1999; Wang et al., 2003; Kızılkaya, 2005, 2008; Delibacak et al., 2009; Candemir and Gülser, 2010; Gülser and Candemir, 2012; Cercioğlu et al., 2014). Nevertheless, it is fundamental to control and limit the environmental impact of these practices since they can result in organic or inorganic contamination of natural resources. Among the pollutants, heavy metals have been critically examined since they can be toxic to humans, animals and plants (Baize and Sterckeman, 2001).

Farmyard manure (FM), which is the most useful organic matter, is provided from various animal wastes. Masood et al., (2014) reported that short-term application of higher FM levels improves soil properties. Furthermore, only the application of FM at higher rates significantly increases the nutrient uptake of maize plants due to improved soil properties. Since FM is not found in sufficient amounts in farms and it is an expensive material, other organic materials can be used instead of manure to improve soil properties and plant nutrients in soil. Tobacco solid waste is classified as an agroindustrial waste. Direct use of tobacco waste could create an unfavorable soil environment; however, composting tobacco waste could accelerate the breakdown of nicotine and result in the production of a less toxic and more useful organic amendment (Adediran et al., 2004).

Turkish soils are known to be widely deficient in N, P and trace elements, and have low rates of organic matter (OM). Therefore, OM and macronutrients and micronutrients concentrations of Turkish soils can be increased by using CTW. In the present study, composted tobacco waste (CTW) combined with FM at different ratios was applied to soil, and the influence of these amendments on the yield and nutrient composition of butter head lettuce (*Lactuca sativa* L.) were investigated.

Material and Methods

Experimental site

The experiment was conducted at the Agriculture Faculty's Research Farm of Ege University in Menemen plain, Izmir, Turkey (38°58′35.51″-38°58′36.03″N; 27°03′84.56″-27°03′89.81″E). The experimental site is in the Western Anatolia region of Turkey, where the Mediterranean climate prevails with a long-term mean annual temperature of 16.9°C. Long-term mean annual precipitation is 536.8 mm, representing about 75% of rainfalls during the winter and spring, and the mean relative humidity is 57%. Long-term mean annual potential evapotranspiration is 1570 mm (IARTC, 2012). The investigated soil is characterized by loam texture with slightly alkaline reaction and classified as a Typic Xerofluvent (Soil Survey Staff, 2006). Some physical and chemical properties and macronutrients and micronutrients in the experimental soil are given in Table 1.

Table 1. Some physical and chemical properties of the experimental soil (0-30 cm)

Parameter	Value
Soil texture	Loam
Sand (%)	44.26
Silt (%)	44.13
Clay (%)	11.61
pH	7.52
Total soluble salt (%)	0.085
CaCO3 (%)	5.38
Organic matter (%)	2.53
Total N (%)	0.129
Available P (mg kg^{-1})	8.88
Available K (mg kg^{-1})	447.2
Available Ca (mg kg^{-1})	2752
Available Mg (mg kg^{-1})	529.4
Available Na (mg kg^{-1})	217.9

Field experiment

The experiment was conducted in 18 parcels in a randomized-block design with three replications. The parcel size was 3×2 m. The organic materials were CTW and FM. The general properties of the organic materials are given in Table 2. The treatments were (1) control, (2) 12.5 t ha^{-1} FM + 37.5 t ha^{-1} CTW, (3) 25 t ha^{-1} FM + 25 t ha^{-1} CTW, (4) 50 t ha^{-1} FM, (5) 50 t ha^{-1} CTW, and (6) 37.5 t ha^{-1} FM + 12.5 t ha^{-1} CTW. Tobacco waste was taken from the Izmir Kemalpasa Socotab Cigarette Factory, and FM was obtained from the

Agriculture Faculty's Research Farm, Ege University, Menemen. Both materials were applied to the soil after composting. At the beginning of the experiment, 50 t ha^{-1} materials were applied to the soil because lettuce plants need 50-100 kg N ha^{-1} (IFA, 1992). Five hundred forty lettuce seedlings were planted in the first vegetation period by furrow irrigation. After that, irrigation method was changed to drip irrigation. The first harvest was made by hands. Similarly, during the second vegetation period, five hundred forty lettuce seedlings were planted by irrigation, and they were not irrigated until the end of the harvest. The second harvest was performed again by hands.

Table 2. Some properties of composted tobacco waste (CTW) and farmyard manure (FM)

Parameter	CTW	FM
pH	9.17	8.70
EC (dSm^{-1})	40	38.5
Org. C (%)	37.87	39
OM (%)	65.3	67.2
C/N	17.37	16.5
CaCO3 (%)	2.43	2.09
60 ◦C water content (%)	7.19	5.50
105 ◦C water content (%)	29.79	25.13
Total N (%)	2.18	2.35
Total P (%)	0.49	0.58
Total K (%)	2.688	3.072
Total Ca (%)	1.287	1.521
Total Mg (%)	0.655	0.615
Total Na (%)	0.255	0.281

Soil and plant sampling and analyses

During the experiment, soil samples (0-30 cm) were taken from the center of each parcel after one week of planting and before first (I) and second (II) harvests. The samples were air dried and sieved through 2 mm sieve. Particle-size distribution was determined according to Bouyoucos (1962). Gravimetric water content of moist FM and CTW were determined according to Jury et al. (1991). Total salt, OM concentration, $CaCO_3$, pH, total N, P, K, Ca, Mg, Na, Fe, Cu, Mn and Zn, concentrations of soil and CTW and FM and butter head lettuce samples were all determined according to Page et al. (1982). Available P was determined by the Mo blue method in a $NaHCO_3$ extract (Olsen et al., 1954). Available Ca, Mg, K and Na were analyzed with 1N NH_4OAc extract method. Ca, K and Na were determined by flame emission spectrometry and Mg was determined by flame atomic absorption spectrometry (AAS) (Kacar, 1994). Fe, Mn, Zn and Cu were extracted using DTPA (diethylene triamine pentaacetic acid) solution (Lindsay and Norwell, 1978). The concentrations of these elements in the extracts were determined by AAS (AOAC, 1990).

Statistical Analysis

Analysis of variance (ANOVA) was performed using the Statistical Package for the Social Sciences, version SPSS 17.0. Treatment differences between mean values of parameters were evaluated by one-way ANOVA followed by Duncan's test of significance at $P \leq 0.05$ (SPSS 17.0, 2008).

Results and Discussion

Influence of CTW and FM applications on grown lettuce yield in Typic Xerofluvent soil

Influence of CTW and FM applications on grown lettuce yield in Typic Xerofluvent soil are given in Table 3.

Table 3. Influence of composted tobacco waste (CTW) and farmyard manure (FM) applications on the lettuce yield

Treatments	Lettuce yield (t ha^{-1})		
	First harvest	Second harvest	Total yield
Control	50.7 b	31.0 c	81.7 b
25% FM+75% CTW	60.8 a	38.0 ab	98.8 a
50% FM+50% CTW	59.9 a	37.7 ab	97.6 a
100%FM	60.9 a	37.4 ab	98.4 a
100% CTW	62.7 a	39.9 a	102.7 a
75% FM+25% CTW	60.1 a	36.0 b	96.2 a

Means for CTW and FM rates applied in soil in the same period followed by the different letters are significantly different (Duncan; P ≤ 0.05)

In accordance with the controls, CTW and FM applications have increased the yield of lettuce statistically. The maximum yield was obtained during the 1st vegetation period (62.7 t ha^{-1}) in the 100 % CTW application. On account of the 2nd vegetation period's coinciding with winter and the coldness of the months December, January and February, there happened a slowdown in the lettuce yield. The highest total yield of lettuce in both vegetation periods (102.7 t ha^{-1}) was determined in 100% CTW application parcels. The lower lettuce yields were determined in the control parcels at the 1st and 2nd harvest as 50.7 and 31.0 t ha^{-1} respectively. Therefore the lowest total yield was obtained in the control as 81.7 t ha^{-1} (Table 4). Gunes et al. (2014) reported that application of biochar and poultry manure to soil significantly increased lettuce growth.

Table 4. Influence of composted tobacco waste (CTW) and farmyard manure (FM) applications on the mineral composition of lettuce

Applications	In the samples of 1st lettuce harvest	In the samples of 2nd lettuce harvest	In the samples of 1st lettuce harvest	In the samples of 2nd lettuce harvest
	N (%)		P (%)	
Control	2.35 c	2.74 b	0.57 b	0.61 b
25% FM+75% CTW	3.08 a	3.18 a	0.73 a	0.71 a
50% FM+50% CTW	3.01 ab	2.98 ab	0.68 a	0.72 a
100%FM	2.89 ab	3.38 a	0.71 a	0.73 a
100% CTW	3.01 ab	3.08 ab	0.66 a	0.70 a
75% FM+25% CTW	2.62 b	3.22 a	0.68 a	0.73 a
	K (%)		Ca (mg kg^{-1})	
Control	7.42 ab	4.60 a	7049 d	5506 e
25% FM+75% CTW	7.86 a	4.98 a	7550 c	6433 cd
50% FM+50% CTW	7.93 a	5.07 a	7906 b	6506 bc
100%FM	7.97 a	5.35 a	8125 a	6836 a
100% CTW	7.67 ab	4.91 a	7932 ab	6320 d
75% FM+25% CTW	7.90 a	5.19 a	7963 ab	6676 ab
	Mg (mg kg^{-1})		Na (mg kg^{-1})	
Control	2102 b	1318 c	4179 c	1334 b
25% FM+75% CTW	2187 ab	1585 ab	4383 b	1429 ab
50% FM+50% CTW	2353 a	1452 abc	4424 ab	1477 a
100%FM	2183 ab	1418 bc	4519 a	1522 a
100% CTW	2300 ab	1652 a	4343 b	1432 a
75% FM+25% CTW	2228 ab	1452 abc	4419 ab	1499 a
	Fe (mg kg^{-1})		Cu (mg kg^{-1})	
Control	145.8 c	82.6 c	10.4 d	12.6 c
25% FM+75% CTW	192.4 ab	106.2 ab	18.1 bc	18.9 b
50% FM+50% CTW	183.3 b	101.2 ab	18.9 abc	21.3 a
100%FM	178.5 b	92.6 bc	19.8 a	21.0 a
100% CTW	207.2 a	112.9 a	18.0 c	19.1 b
75% FM+25% CTW	182.0 b	97.2 abc	19.6 ab	20.3 ab
	Zn (mg kg^{-1})		Mn (mg kg^{-1})	
Control	16.0 c	26.6 d	33.1 b	24.5 b
25% FM+75% CTW	21.3 ab	35.3 a	38.5 a	26.7 ab
50% FM+50% CTW	21.2 ab	32.3 abc	38.4 a	26.8 ab
100%FM	18.3 bc	30.3 c	39.8 a	27.2 a
100% CTW	24.2 a	33.6 ab	39.2 a	25.3 ab
75% FM+25% CTW	19.2 b	30.6 bc	39.1 a	26.4 ab

Means for CTW and FM rates applied in soil in the same period followed by the different letters are significantly different (Duncan; P ≤ 0.05)

Influence of CTW and FM applications on the nutrient composition of lettuce

Influence of CTW and FM applications on the nutrient element composition of lettuce were given in the Table 4. N content of the lettuce samples showed an increase in both periods statistically with CTW and FM applications. Minimum N content was determined in the plant samples that were taken from the control parcels. While the maximum value was obtained in 25% FM+75% CTW applications with 3.08% N, the minimum value was determined in the control parcels as 2.35 % N. Tepecik et al. (2014) found that total N contents varied between 2.11 % and 2.70 % in the conventionally grown basil and between 2.21 % and 2.39 % in the organically grown basil.

P content of the lettuce in both period samples showed an increase with CTW and FM applications. Statistically, while the control was in a separate group with the lowest P content, other applications took place within the same group. Gunes et al. (2014) determined that phosphorus concentration of the lettuce leaves significantly increased by poultry manure and biochar treatments.

K content of the lettuce samples of the first period showed increase with CTW and FM applications. Besides that, while statistically the control and 100% CTW applications are found in the same group with the minimum K contents (7.42%; 7.62%), the maximum K content was determined 100% FM application (7.97%) and other applications took place in different group. Gunes et al. (2014) reported that Lettuce K concentrations were increased in response to poultry manure and biochar.

The effect of 100% FM application on Ca content of lettuce samples showed the highest increase in both periods (8125-6836 mg kg^{-1}) and took place in a different group statistically. Minimum Ca quantities among all applications were obtained from control samples (7049-5506 mg kg^{-1}).

The highest Mg quantity in the first period lettuce samples was determined in 50% CTW+50% FM application (2353 mg kg^{-1}). On the other hand, the highest Mg quantity in second period lettuce samples was (1652 mg kg^{-1}) in 100% CTW application and these applications statistically took place in different groups. The minimum Mg quantities in both periods were determined in control parcels (2102-1318 mg kg^{-1}) and statistically took place in different group from other applications.

While the highest Na quantity of the lettuce samples in the first period was determined in 100% FM application (4519 mg kg^{-1}), the minimum Na quantity was found (4179 mg kg^{-1}) in control samples. In second period lettuce samples, the Na quantity in lettuce was generally decreased. The Na quantity which was minimum in the control with 1334 mg kg^{-1} meanwhile the maximum quantity of Na was determined as 1522 mg kg^{-1} with 100% FM application. The Na content of the second period lettuce samples was low stemmed from the fact that no irrigation was done because the amount of rainfall in this period was high which caused the washing of Na in the soil. The Na content of first period samples was determined as higher than the second period samples due to the Na that came from irrigation water and applications.

Maximum Fe quantity of lettuce samples in both periods was determined in 100% CTW aplication (207.2-112.9 mg kg^{-1}) and this aplication statistically took place in different group distinct from control and other aplications. As for the minimum Fe quantity was determined again in the lettuce samples that were taken from control parcels (145.8-82.6 mg kg^{-1}).
When Cu contents of the lettuce samples were examined, the maximum value in the first period was obtained in 100% FM application with 19.8 mg kg^{-1} and in the second period, they were obtained in 100% FM (21,0 mg kg^{-1}) and 50% CTW+50% FM (21.3 mg kg^{-1}) applications. Cu quantity of both periods was found minimum in the control samples and the control took place statistically in a different group.

The maximum Zn content of the first period lettuce samples was determined in the 100% CTW application (24.2 mg kg^{-1}) and statistically this application took place in a different group from control and other applications. As for the second period lettuce samples, the maximum Zn value was determined in the 25% FM+75% CTW application (35.3 mg kg^{-1}). The minimum Zn quantities were found in the control samples in both periods (16.0-26.6 mg kg^{-1}).

Mn content of lettuce samples increased according to the control in the first period and while all of the applications took place statistically in the same group, the control samples took place in different group. Maximum Mn content of first and second period samples was determined in 100 %FM application as 39.8-27.2 mg kg^{-1}, respectively.

In a study about lettuce with different organic fertilizers, Demir et al. (2003) reported that average Mg, Na, Fe, Cu, Mn and Zn content of lettuce were determined as mg kg^{-1} 2321, 1007, 172.39, 12.51, 61.99 and 45.33, respectively. The quantitites of nutritional elements in the lettuce samples apart from N, P, Cu, Zn and Mn were determined as much higher in the first period products than the second period products. This case is also the same with lettuce yield. The reason behind this can be attributed to the fact that the second period lettuce production happened in winter and metabolic activities showed a decrease because of the decrease of the temperatures.

Conclusion

CTW and FM applications raised the lettuce yield and N, P, K Ca, Mg, Na, Fe, Zn and Mn contents of the lettuce. According to the results obtained, it can be said that CTW can be used in agricultural fields just like FM. Especially the 25%+75% CTW and 100% CTW applications showed more important effects. These results indicate that CTW can function as an alternate organic additive (soil improver) so as to enhance the amount of organic matter in the soils of Aegean Region that has the Mediterranean climate and contain low amount of organic matter. It is useful to apply tobacco wastes on soil by composting them because of their high content of nicotine. For the applications without composting, it is suggested to wait for at least a month for sowing-planting process after the application. Some other studies that will reveal the positive, long-term affects of CTW should also be carried out so as to protect and enhance the soil quality.

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Water stress and soil compaction impacts on clover growth and nutrient concentration

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Abstract

Soil compaction and insufficient water supply generally decrease crop performance. The effects of varying compaction and water availability levels on the growth of Berseem or Egyptian clover (*Trifolium alexandrimum* L.), water use efficiency and nutrient concentration were investigated under greenhouse conditions. Treatments consisted of three soil compaction levels (bulk density of 1.2, 1.4 and 1.6 Mg m^{-3}), and four water availability treatments (40%, 60%, 80% and 100% of soil field capacity) in a factorial combination. Soil compaction had a significant effect on water use efficiency with the highest (0.32 g l^{-1}) at bulk density of 1.4 Mg m^{-3} and the lowest at the other bulk densities. Soil compaction had no significant effects on leaf area, shoot, root and total dry masses. Water stress resulted in lower leaf area (from 231 to 153 mm^2 pot^{-1}), and the stem lengths were 7.6 cm and 4.3 cm for 80% and 60% of field capacity, respectively. Likewise, the highest (0.47 g pot^{-1}) and lowest (0.33 g pot^{-1}) total dry masses were observed at 80% and 60% field capacities. Water use efficiencies were 0.32 and 0.20 g l^{-1} for 100% and 60% field capacities, respectively. The accumulation of N, P and K per unit length of roots increased with soil compaction. As the water supply increased, the root and shoot dry weight and water use efficiency increased. Treatment of 100% field capacity resulted in the highest accumulation of N, P and K. Results indicated that the treatment of 80% field capacity and bulk density of 1.4 Mg m^{-3} provided the best conditions for clover performance, among the applied treatments. This study suggests that sufficient water supply can moderate the adverse effects of soil compaction on clover performance.

Keywords: Water stress, compaction, water use efficiency, clover growth, nutrient concentration

Introduction

Water scarcity is very pronounced because of meteorological condition, rising population, mismanagement of soil and water resources and the global warming due to climate change. The impact is severe in arid and semi-arid regions where the soil mechanical impedance and water stress are among the most environmental constrain for crop growth.

Soil compaction often alters soil physical properties including water infiltration and distribution, gaseous movement, and nutrient uptake resulting in changes in root elongation and plant-available water. The ability of roots to penetrate strong soil has been studied (Barzegar et al. 2006). The response of roots to soil

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physical constrains was comprehensively reviewed by Bengough et al. (2011). Physical, biological and chemical processes of the root zone have been reviewed (Gregory, 2006; Hinsinger et al., 2009).

Soil degradation due to induced compaction affects about 68 million hectares worldwide (Flowers and Lal, 1998). The severity of soil compaction depends on several factors including soil type (e.g. soil texture and structure), soil water content, machinery properties (e.g. weight, speed, and contact area of tire and soil surface), and farming practices (Chamen et al., 2003). Grzesiak et al. (2013) indicated that soil compaction decreased leaf area, and biomass of shoots and roots.

Taylor (1983) reported that soil water potentials greater than -1 MPa do not have a direct impact on root growth; as soils dries out, resulting in lower soil water potential and causing strength to increases rapidly. Whalley et al. (2005) indicated that higher effective stress between soil particles resulted in lower root growth. Some reports indicated that as the soil water indirectly impacts other soil properties including soil penetration resistance, aeration, composition of soil solution, it would be difficult to isolate soil water impact on root growth. However, some evidence suggested lower root growth at low water potential due to hormonal changes of root (Blum, 2011). The influence of water stress on crop performance may be exacerbated by increased soil compaction associated with heavier farm machinery (Bengough et al., 2006). Bengough et al. (2011) reported that the response of root elongation rate decreases in response to both increasing soil impedance and decreasing matric potential but may vary among different crops.

The impact of water stress on crop growth depends upon the intensity and duration of drought, growth stage, the genotype and physiology of the crop species. Whitmore and Whalley (2009) reviewed the impact of soil drying on root and crop growth and suggested that drought is not a single, simple stress and that agronomic practices need to take into account the multiple facts of both the stress caused by insufficient water along with other interacting stresses such as heat, disease, soil strength and low nutrient status.

This study was conducted to investigate the growth performance of clover under different water stress conditions and various compaction treatments and determine whether water stress increases the negative impact of soil compaction on clover growth and nutrient uptake.

Material and Methods

Soil preparation

A Typic Torrifluvent (USDA), Calcaric Fluvisols (FAO) was used in this experiment. Soil was mixed thoroughly and sieved to remove stones and debris before pouring it into pots, measuring 12 cm height and 10 cm diameter. A subsample of soil was used to measure chemical and physical properties.

Electrical conductivity of a saturated extract (Rhoades, 1982) and pH of a saturated paste were determined. Organic carbon was measured by wet oxidation (Nelson and Sommers, 1982). Particle size distribution was determined by the pipette method (Gee and Bauder, 1986). Water contents at field capacity (-33 kPa suction) and permanent wilting point (-1500 kPa suction) were estimated using a pressure plate apparatus. Soil analysis showed a pH of 7.7, organic matter of 7.7 g kg^{-1}, EC of 1.8 dSm^{-1}. Water contents at field capacity and permanent wilting point were 210 and 90 g kg^{-1}, respectively. The soil had 326 g kg^{-1} clay, 474 g kg^{-1} silt and 200 g kg^{-1} sand. The optimum soil water content was determined using the standard Proctor test (American Society for Testing and Materials, 1992). Soil was moistened at different levels of water contents. Samples were compacted by dropping a 2.5 kg hammer 75 times from a height of 30 cm, and soil bulk density was determined.

Experimental design

A 4×3×3 factorial randomized block experimental design was performed. Treatments included three soil compaction levels, i.e., bulk density of 1.2, 1.4 and 1.6 Mg m^{-3} and four watering treatments, i.e. 40%, 60%, 80% and 100% of field capacity. There were 36 treatment combinations in total replicated 3 times. Preparation of each pot was carried out using the method outlined by Barzegar et al. (2006). After mixing the amount of water required for optimum compaction in a plastic bag the soil sample was transferred to a pot. The soil was poured in four layers of 5 cm increments into a pot mounted on a hydraulic jack and compacted. An increasing load was applied in steps for a short time to obtain the desired bulk density.

Seeds of Berseem or Egyptian clover (*Trifolium alexandrimum* L.) were pre-germinated for 72 h at 25˚C on wetted filter paper. Six seedlings were planted in each pot and covered by a 2 cm layer of uncompacted soil. The watering regime of pots consisted of weighing each pot once a day and adding water to the weight corresponding to 70% of the field capacity. Two weeks after transplanting, the number of seedlings in each pot was reduced to three, and the watering treatments were implemented. After 8 weeks, the shoots and roots of each treatment were collected.

Plant measurements

Roots were separated from the soil by washing. Subsamples of roots and shoots were dried for 72 h at 60–65˚C and dry mass determined. Total leaf area was measured with a leaf area meter. Wet and dry weight of both roots and shoot biomass were determined. Water use efficiency was calculated as the ratio of dry weight of above-ground biomass to water used for irrigation. Length of shoot and roots was recorded. Subsamples of shoots and roots of each treatment were digested separately in 20% nitric acid (Mills and Jones, 1996) for determination of P, K and N concentrations.

Analysis of variance of the data was performed using SPSS to determine the significance of water availability regimes and soil compaction levels and their interactions. Means were separated using the Duncan test.

Results and Discussion

Crop growth

Clover did not grow at 40% of F.C for all the compaction treatments; therefore, we presented the results from the other water availability treatments (e.g. 06FC, 0.8FC and FC). The lack of growth at 0.4FC in this study is in contrast with the results reported for tomato by Nahar and Gretzmacher (2002). This inconsistency may be due to differences in crop response to low soil water potential and also can be contributed to difference in the climatic condition (temperature and humidity). There was a significant interaction between clover performance and soil compaction and water availability treatments (Table 1) for all the variables except root dry matter.

Table 1. Mean values of measured clover parameters, water use efficiency (WUE), and nutrient concentration

Treatment	df	Leaf area, mm^2	Stem length, cm	Total dry mass, g pot^{-1}	Shoot dry mass, g pot^{-1}	Root dry matter, g pot^{-1}	WUE, g lit^{-1}	N, %	P, %	K, %
Replication	2	410.2^{ns}	0.32^{ns}	0.005^{ns}	0.001^{ns}	0.0001^{ns}	0.0002^{ns}	0.04^{ns}	0.0003^{ns}	0.006^{ns}
Compaction(C)	2	1950.6^{*}	3.1^{*}	0.041^{**}	0.021^{**}	0.0002^{ns}	0.025^{**}	45.7^{**}	0.049^{**}	0.826^{**}
Water stress (WS)	2	13899.2^{**}	26.1^{**}	0.053^{**}	0.027^{**}	0.001^{*}	0.038^{**}	1.79^{**}	0.014^{**}	2.58^{**}
C×WS	4	8260.3^{**}	8.5^{**}	0.038^{**}	0.011^{*}	0.0002^{ns}	0.011^{**}	9.4^{**}	0.01^{**}	2.62^{**}
Error	18	560.3^{ns}	0.53^{**}	0.006^{ns}	0.003^{ns}	0.0001^{ns}	0.0001^{ns}	0.02^{ns}	0.0001^{ns}	0.007^{ns}

* Significant P≤0.05 ; ** Significant P≤0.01 ; ^{ns}, Not significant

Increasing compaction level reduced clover leaf area, however, the effect was not significantly different among soil compaction treatments. Water stress reduced the leaf area significantly (P≤0.01) with the highest leaf area at FC (231 mm^2) and the lowest at 0.6 FC (153 mm^2). The interactive effects of soil compaction and water stress on the leaf area were significant (P≤0.05) (Figure 1). Hopkins (2004) reported that water stress adversely influences the photosynthesis systems and results in lower leaf area.

Shoot length was significantly affected by both compaction and water stress and their interactions (P≤0.01) (Figure 1). Shoot length of bulk density of 1.2 and 1.6 Mg m^{-3} treatments were 6.5 cm and 5.5 cm, respectively. The highest (7.6 cm) and lowest (4.2 cm) shoot length were obtained at water stress level of 0.8 and 0.6FC, respectively. Comparison of mean indicated that the bulk density of 1.2 and 1.4 Mg m^{-3} had the highest and 1.6 the lowest shoot length respectively, whereas the water stress at 0.8 FC had the highest shoot length. Plaut (2008) suggested that soil physico-chemical degradation by induced compaction in arid and semi-arid regions results in reducing rhizosphere biological activities and lower water and nutrient concentration by crops.

The dry mass of roots, shoots and whole crop was not significantly affected by soil compaction level. However, water stress level significantly (P≤0.01) impacted the dry mass weights with the highest at 0.8FC and the lowest at 0.6FC. The 100 % FC probably reduced oxygen availability, due to excess of water, and in contrast, at 40 % FC the plants do not get enough water for physiological functioning. The 80 % FC gave the highest yield. Similar results were reported for tomatoes where the highest yield was at 70% FC and the 100% and 40% FC resulted in lower yield (Nahar and Gretzmacher, 2002).

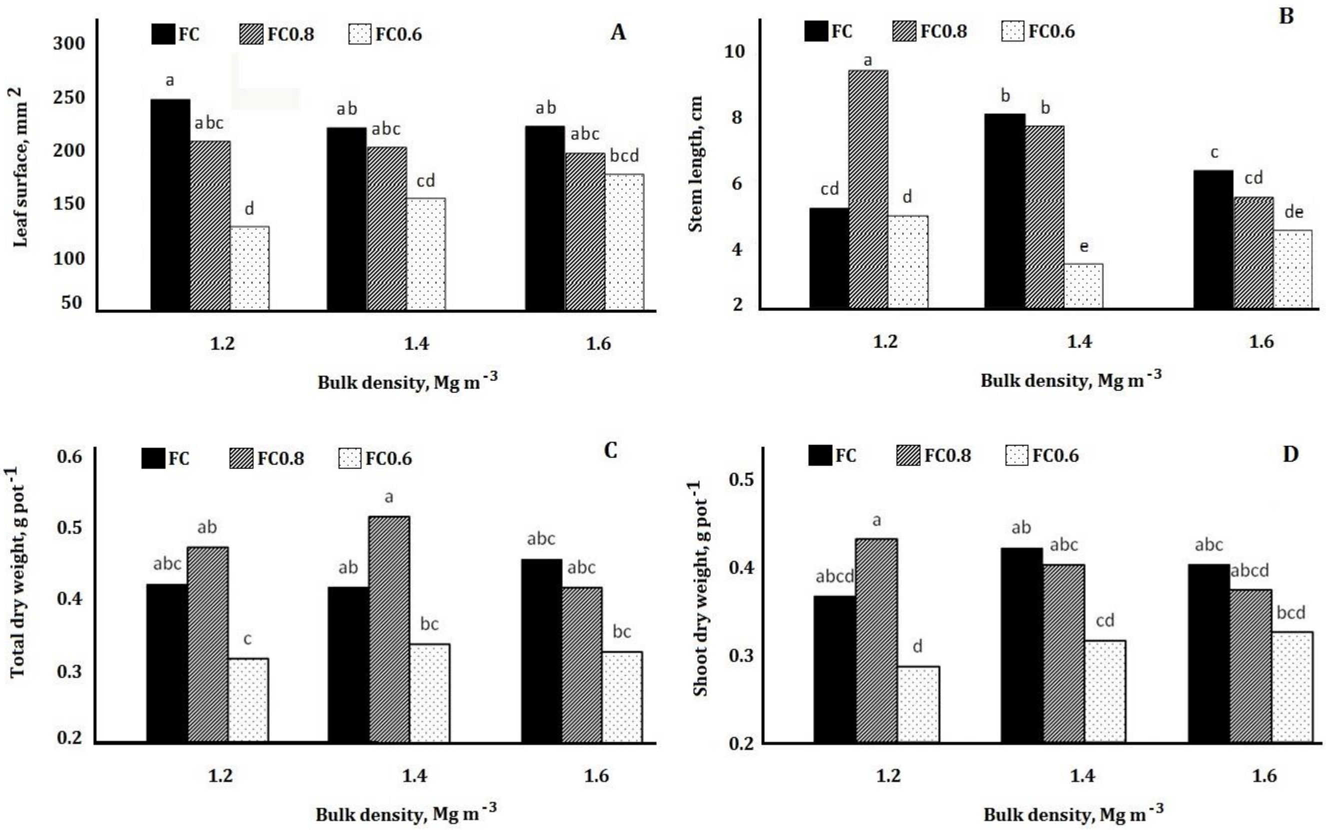

Figure 1. Leaf are (a), Stem length (b), total (c) and shoot (d) dry matter weight of clover as affected by different levels of soil compactions and water sress leve; means with similar letters are not significantly different.

Water use efficiency

The effect of soil compaction levels on water use efficiency (WUE) was significant (P≤0.01). All main effects and their two- way interactions for shoot dry mass and root length were significant. The highest WUE (0.32 g l^{-1}) was found at bulk density of 1.4 Mg m^{-3} and decreased (0.23 g l^{-1}) for bulk density either of 1.2 or 1.6 Mg m^{-3} (Figure 2). The water stress level had significant effects on WUE. The interactive effect of soil compaction levels and water stress revealed the highest WUE (0.38 g l^{-1}) was at bulk density of 1.4 Mg m^{-3} and 100% FC, and the lowest WUE (0.17 g l^{-1}) was obtained with treatment of bulk density of 1.2 Mg m^{-3} and 60% FC. In highly compacted soils oxygen availability is a limiting factor for root growth (Arvidsson, 1999). Lipiec et al. (2003) reviewed the impact of soil compaction on root growth and crop yield in Europe and suggested that an increase in soil compaction results in decreased root size, the higher concentration of roots in the upper soil, lower rooting depth and a greater distance between the nearest roots. Insufficient water supply decreased in compacted soil whereas the efficiency of the use of water by the roots increased.

Nutrients concentration

Soil compaction increased the N uptake by clover. The highest N accumulation (6.9%) was associated with the bulk density of 1.6 Mg m^{-3} and the lowest (2.4%) at 1.2 Mg m^{-3}. Also the highest N concentration was at the 100% F.C. treatments followed by the other water stress treatment (Figure 3). The nitrogen loss in soil is mainly by mass flow of water through the macropores. The higher the bulk density the lower the macropores and lower nitrogen loss due to leaching. The interaction effect of both soil compaction and water stress levels was significant (P≤0.01) and the highest N concentration was at bulk density of 1.6 Mg m^{-3} 100% FC. The results are consistent with those reported by others (e.g. Nahar and Gretzmacher, 2002). Likewise water stress also significantly influenced the concentration of phosphorus and potassium in a similar way to that of N. The mean compare of K and P shows significant difference among treatments. The treatment of bulk density of 1.6 Mg m^{-3} 100% FC indicates the highest P and K concentration of 0.3 and 4.2%, respectively. Logan et al. (1997) reported similar trends for vegetables under water stress condition.

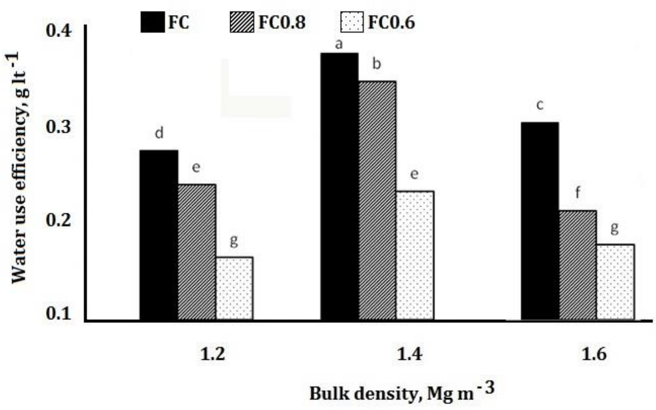

Figure 2. Water use efficiency of clover under diffent levels of water stress and soil compactions; means with similar letters are not significantly different.

Soil compaction results in lower nutrient concentration by plants. Barzegar et al. (2006) indicated the lower P, and Zn by clover as the soil compaction level increases. Similarly, Rahman et al. (2005) showed that Cu, N, P, K, Fe, Mn and Zn concentration by plant decreased as the soil compaction increased. Our results are consistent with those reported by Lipiec et al. (1991; 2003). They indicated that both nutrient concentration and effectiveness of fertilization is reduced by soil compaction. Bharameh and Josh (1993) reported that the concentration of N, P, K, Ca and Mg by sorghum was adversely affected under the irrigation treatments of decreasing soil water potential below field capacity. Our results indicated a tendency to diminish concentrations of N, P and K when increasing water stress (Figure 3).

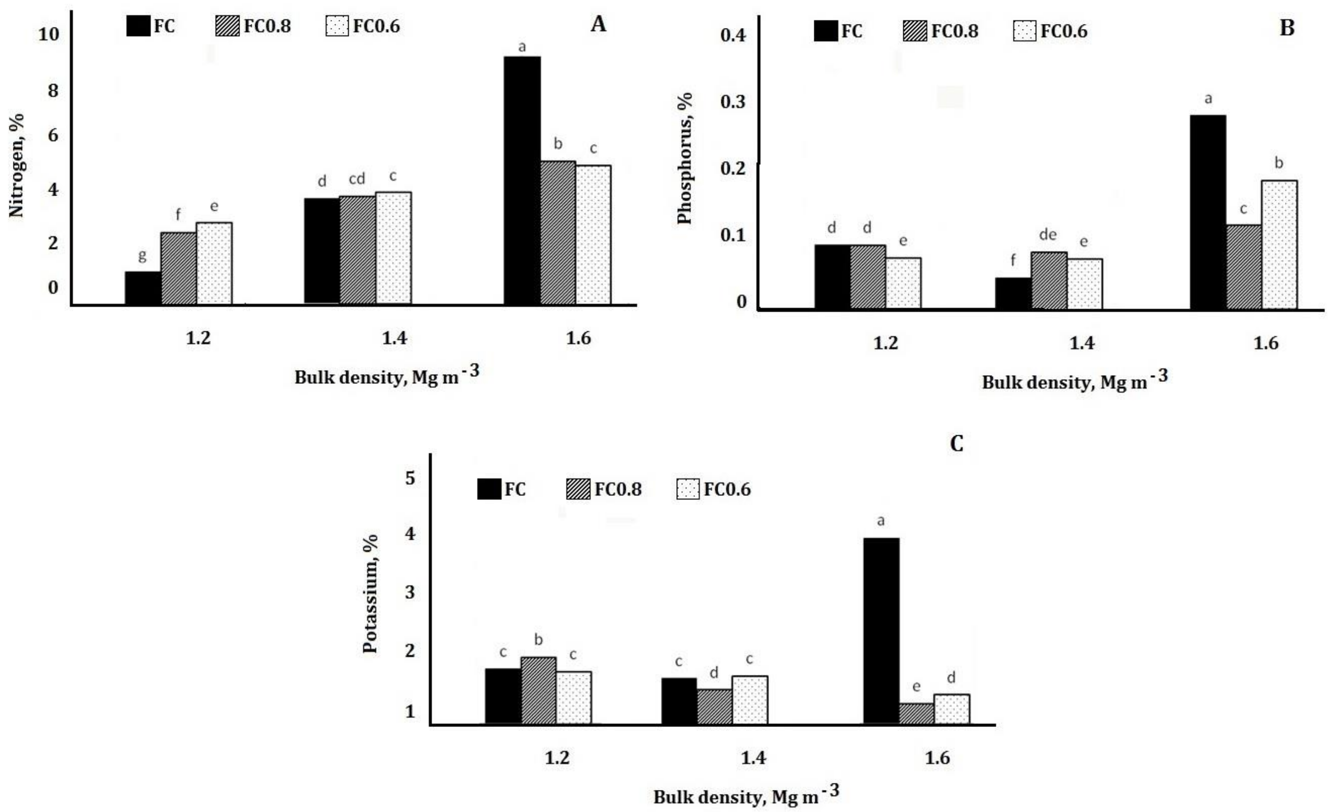

Figure 3. Inflence of diffent levels of water stress and soil compactions on N (a), P (b) and K (c) concentration by clover; means with similar letters are not significantly different.

Conclusion

An increase in soil compaction and insufficient irrigation water reduced shoot and root dry mass, and water use efficiency of clover. Soil water content at or near field capacity resulted in higher water use efficiency and nutrient concentration by clover and higher yield even at higher soil compaction.

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Carbon dioxide emission and soil microbial respiration activity of Chernozems under anthropogenic transformation of terrestrial ecosystems

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Abstract

The total soil CO_2 emission (EM) and portion of microbial respiration were measured (*in situ*; May, June, July 2015) in Chernozems typical of virgin steppe, oak forest, bare fallow and urban ecosystems (Kursk region, Russia). In soil samples (upper 10 cm layer), the soil microbial biomass carbon (C_{mic}), basal respiration (BR) and fungi-to-bacteria ratio were determined and the specific microbial respiration (BR / C_{mic} = qCO$_2$) was calculated. The EM was varied from 2.0 (fallow) to 23.2 (steppe) g CO_2 m^{-2} d^{-1}. The portion of microbial respiration in EM was reached in average 83, 51 and 60% for forest, steppe and urban, respectively. The soil C_{mic} and BR were decreased along a gradient of ecosystems transformation (by 4 and 2 times less, respectively), while the qCO$_2$ of urban soil was higher (in average by 42%) compared to steppe, forest and fallow. In urban soil the C_{mic} portion in soil C_{org} and C_{fungi}-to-C_{org} ratio were by 2.6 and 2.4 times less than those for steppe. The relationship between microbial respiration and BR values in Chernozems of various ecosystems was significant (R^2 = 0.57).

Keywords: Chernozems, CO_2 emission, ecosystem land use, microbial biomass, microbial respiration

Introduction

The circulation of carbon dioxide between soil and atmosphere provided by two main processes: plants photosynthesis and respiration of soil. Soil respiration (soil CO_2 emission), in turn, is provided by respiration of soil microorganisms and plant roots. It is believed that about 70% of total soil CO_2 emission was derived by soil microbial respiration (Zavarzin and Kudeyarov, 2006). According to many researchers the portion of respiration of soil microorganisms and plant roots in total soil CO_2 emission depends on hydrothermal conditions (Ryan and Law, 2005; Martin and Bolstad, 2005), photosynthesis activity (Kuzyakov and Gavrichkova, 2010) and soil organic matter composition (Metting, 1993), but it mainly remains still unclear (Hanson et. al, 2000; Kuzyakov and Larionova, 2005). In addition, the separation of these soil CO_2 fluxes in

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natural condition is difficult to defined (Kuzyakov and Larionova, 2005) and time- and labor-consuming (Yevdokimov et al., 2010). Moreover, the determination of these two portions in total soil CO_2 emission might be very important for studying carbon cycle and modeling carbon change in terrestrial ecosystems (Wie et al., 2010).

Chernozems is an important natural resource of Russia, the distribution of Chernozems area is about 6% of the country (National Soil Atlas..., 2011). Plowing Chernozems is currently reached up 50-60%, which made almost 2 / 3 of all agricultural production in Russia. For the last 150 years the carbon content of Chernozems was decreased by 20-30% (Mikhailova and Post, 2006). Besides, the urban area in Chernozems zone increases by 10% for 2010-2015 yrs (Statistical Pocketbook, 2015).

Anthropogenic transformation of terrestrial ecosystems (agricultural use, urbanization) leads to the changes of soil microbial community functioning. It was shown that in arable Chernozems the soil microbial biomass content was dramatically decreased by almost 3-4 times (Senicovscaia, 2012; Ivashchenko et al., 2015). In Chernozems of Voronezh region (Russia) the soil microbial biomass carbon and its portion in total soil organic carbon were decreased by 2 times and by 40%, respectively, compared to natural analogue (Blagodatskii et al., 2008).

Our study was focused on: i) the measurements of total CO_2 emission from Chernozems and portion of soil microbial respiration *in situ* in natural and anthropogenically transformed ecosystems; ii) the parameters estimation of soil microbial community functioning (soil microbial biomass carbon content, basal respiration, specific respiration of microbial biomass and fungi-to-bacteria ratio); iii) the assessment of relationship between soil microbial respiration measured *in situ* and laboratory conditions.

Material and Methods

Location

The Chernozems typical (Kursk region, Russia: 51°33′50″-51°39′40″ N / 36°04′58″-36°07′41″ E) of natural (virgin steppe, oak forest) and anthropogenically transformed (bare fallow, urban) ecosystems was studied. The steppe, forest and fallow are located in the Central-Chernozemic State Biosphere Nature Reserve area (12 km from Kursk city), urban ecosystem is an industrial zone of Kursk city (near the factory "Kurskrezinotechnika").

Field measurement

The total soil CO_2 emission was measured (closed-chamber, LI-820) in five spatially distant points on the plot (20 × 20 m) of each ecosystem (ground vegetation cut) and expressed as g CO_2 m^{-2} d^{-1} (20 totally). In each point of the ecosystem the soil temperature and soil moisture were recorded at 10 cm depth. The measurements were carried out in early May, June and July, 2015 yr. Soil samples were taken from 10-cm layer for chemical and microbiological (60 totally) analyzes.

Soil microbial respiration *in situ* was determined by substrate induced respiration method (Larionova et al., 2006; Yevdokimov et al., 2010). Into soil steppe, forest and urban the four "collar-base" were cut in 10-cm depth on the distance 1-2 m from points for total CO_2 emission measurement (Figure 1). In two "collar-base" it was unsieved soil (with roots), in the other two it was sieved soil (mesh 3 mm, roots excluded). The measurement of soil CO_2 from the surface of four "collar-base" started not earlier than in half an hour. The water or glucose solution was added (slow penetration) into unsieved and sieved soils of "collar-base" then. In preliminary experiments it was found that the volume (water or glucose solution) provided slow penetration of liquid through 10 cm soil layer was equaled 0.6, 0.9 and 1.0 L for forest, steppe and urban, respectively. The glucose concentration provided the highest initial soil substrate-induced respiration (Anderson and Domsch, 1978) was amounted 5 mg g^{-1} soil in our experiments. The time between addition of liquid to soil and soil CO_2 measuring was 4 h (preliminary experiment).

Soil microbial respiration (MR) of unsieved (with roots) and water-moistened soil was calculated according by following:

$$MR = (GL - W)_{UNS} \Big/ \left(\frac{GL}{W}\right)_S \times \frac{(GL - W)_{UNS}}{(GL - W)_S}$$

where MR is soil microbial respiration, g CO_2 m^{-2} d^{-1}; GL is CO_2 emission from enriched glucose soil, g CO_2 m^{-2} d^{-1}; W is CO_2 emission from soil with water, g CO_2 m^{-2} d^{-1}; UNS is unsieved soil (with roots); S is sieved soil (without roots).

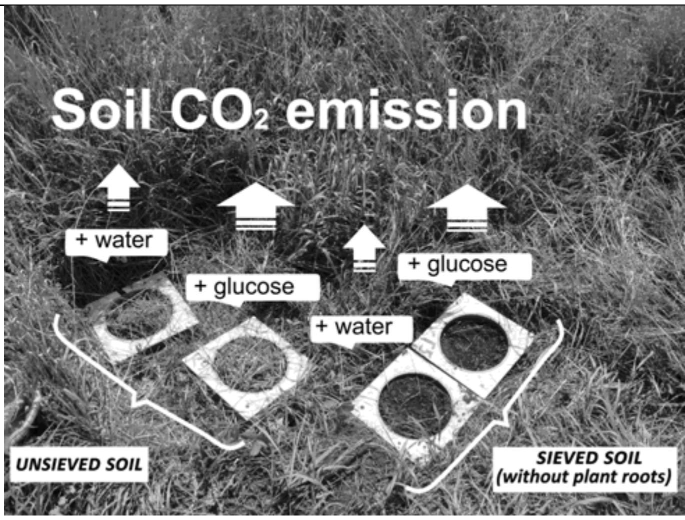


Figure 1. Design of soil microbial respiration measurement in field conditions

The $(GL/W)_S$ ratio was characterized the excess of CO_2 emission from sieved soil added glucose compared to soil added water. The $(GL-W)_{UNS}$ / $(GL-W)_S$ ratio was characterized the soil disturbance (sieving). The MR portion in total CO_2 emission from unsieved (with roots) and water-moistened soil was expressed as MR / W_{UNS} ratio (%). Then we calculated the MR portion in total soil CO_2 emission from nearest point without any liquid addition. Soil MR of steppe, forest and urban was measured in two replicates.

Lab measurement

Soil microbial biomass carbon (C_{mic}) was measured by substrate-induced respiration (SIR) method (Anderson and Domsch, 1978; Ananyeva et al., 2011). Briefly, soil subsamples (1 g) were placed into a 15 ml vial, a glucose solution was added dropwise (0.1 ml, 5 mg glucose g^{-1} soil), vial was closed hermetically and time was recorded. The vial was incubated (3-5 h, 22°C) and an air sample was taken by syringe and injected into a gas chromatograph (KristaLLyuks 4000M, thermal conductivity detector) for measuring CO_2 production. The soil C_{mic} (μg C g^{-1} soil) was calculated by SIR (μl CO_2 g^{-1} soil h^{-1}) × 40.04+0.37 (Anderson, Domsch, 1978).

Soil basal (microbial) respiration (BR) was measured as described for SIR, instead glucose the water added (0.1 ml g^{-1} soil) and incubated (24 h, 22ºC). The soil BR rate was expressed in μg CO_2-C g^{-1} soil h^{-1}.

Specific respiration of soil microbial biomass (microbial metabolic quotient, qCO_2) was estimated as the ratio of BR / C_{mic} = qCO_2 (μg CO_2-C mg^{-1} C_{mic} h^{-1}).

Fungi and bacteria contribution to total SIR in steppe and urban soils was determined by selective inhibition technique (Lin and Brookes, 1999; Bailey et al., 2002). Streptomycin sulfate (water solution) and cycloheximide (powder) were added separately and both into soil subsamples (1 g) for the highest SIR inhibition. More details see in the papers (Susyan et al., 2005; Ananyeva et al., 2014). The fungi-to-bacteria ratio was calculated.

Prior to the lab measurements soil samples (0.3-0.5 kg) were sieved (mesh 2 mm), moistened up to 50-60% water holding capacity and pre-incubated in aerated plastic bags at 22°C for 7 days to avoid excess soil CO_2 production after these manipulations (Ananyeva et al., 2008; Creamer et al., 2014).

Statistical analysis

The measurements were performed in three replicates for SIR, BR and fungi-to-bacteria ratio. The results were calculated for dry soil (105°C, 8 h) and expressed as mean ± standard deviation (Excel). Significance of the difference in total soil CO_2 emission, hydrothermal and microbiological soil parameters between ecosystems was tested by one-factor analysis of variance (ANOVA) and Tukey's multiple comparison test. Statistic tests were chosen based on the preliminary analysis: normality distribution of experimental data was checked by Shapiro-Wilk test, variance homogeneity was checked by Levene's test. Relationship between MR and total soil CO_2 emission, microbiological and hydrothermal soil parameters was analyzed by Pearson's correlation coefficient. Relationship between MR and BR was analyzed nonlinear regression. All experimental data was statistically analyzed and visualized (box-plot) using Statistica 10.0 software. A principal component analysis and ordination of experimental data was carried out by PCord 4.27.

Results and Discussion

The soil organic carbon content (C_{org}) of natural ecosystems was by approximately 2 times higher than that of anthropogenically transformed, and the pH value of steppe and forest was by about one unit less than bare fallow and urban (Table 1). The CO_2 emission from Chernozems in various months was ranged from 2.0 (fallow) to 23.2 (steppe) g CO_2 m^{-2} d^{-1}, these values differ by almost an order of magnitude (Table 2). In May the highest average soil CO_2 emission was found in steppe, forest and urban ecosystems, and the lowest value was in fallow, wherein the soil temperature in forest was significantly low and the soil moisture was high compared to other studied ecosystems. In June a significantly high soil CO_2 emission was found in steppe, and the low one was in fallow, the soil of which was significantly high temperature and low moisture. In the warmest month (July) the soil CO_2 emission from fallow was also the lowest (high soil temperature and low soil moisture). During the observed period (May-July) the high soil CO_2 emission was found in steppe, and the low was in fallow (in average 20.3 and 3.6 g CO_2 m^{-2} d^{-1}, respectively), the difference between these values was almost 6 times (Figure 2).

Table 1. Ecosystem, history treatment (HT), soil organic carbon content and soil acidity (C_{org}, pH, respectively, mean, n = 15) of Chernozems typical (Kursk region, Russia)

Ecosystem		HT, yrs	C_{org}, %	pH_W
Natural	Virgin steppe	75	4.9	5.8
	Oak forest	80	4.8	6.2
Anthropogenically transformed	Bare fallow	60	2.0	7.0
	Urban	70	2.3	7.4

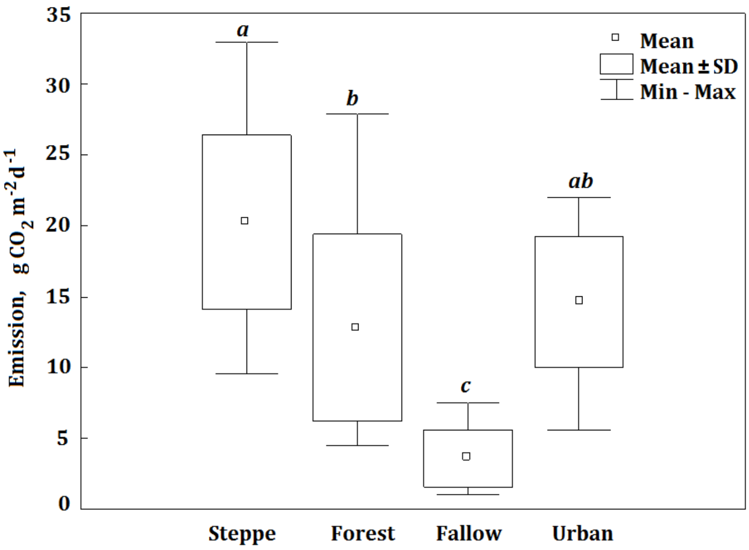


Figure 2. Distribution (box-plot) of CO_2 emission in Chernozems of different ecosystems (n = 15, May-June-July, 2015). The values with different letters were significantly ($p \leq 0.05$) different each other

The soil C_{mic} and BR of various ecosystems for May-June-July were ranged from 284 (urban) to 1710 (steppe) µg C g^{-1} soil and from 0.28 (fallow) to 1.64 (steppe) µg CO_2-C g^{-1} soil h^{-1}, respectively (Table 3). The soil C_{mic} and BR values of steppe and forest were mainly significantly higher than fallow and urban for each studied month. The qCO_2 value in urban soil was on the contrary significantly high in May and June, however in July, the difference of this index for studied ecosystems was not found. For the observed period, the soil C_{mic} content and BR rate of natural ecosystems (steppe, forest) were significantly higher than the anthropogenically transformed (fallow, urban), however the qCO_2 of urban soil was significantly higher compared to other ecosystems (Figure 3). Therefore, there is a base to consider the “deterioration” of soil microbial community functioning in anthropogenically transformed ecosystems compared to natural analogues. In our experiments the highest SIR inhibition by antibiotics both was achieved 41-51% (Table 4). The fungi portion in urban and steppe soils was almost the same (82-85%), wherein the fungi / bacteria ratios were also approximately equal (3.4 and 3.8, respectively). However, the C_{mic} / C_{org} (as an indicator of soil organic matter "quality") and C_{fungi} / C_{org} ratios for urban soil were 2.6 and 2.4 times less than those for steppe. It might be indicated the essential "deterioration" of soil microbial community functioning under anthropogenic impact.

Table 2. Soil CO_2 emission (g CO_2 m^{-2} d^{-1}), soil temperature (T) and soil water content (W) of Chernozems typical in different ecosystems. The values with different letters were significantly (p ≤0.05) differ for each parameter separately

Ecosystem	May			June			July		
(n = 5)	Emission	T,°C	W, %	Emission	T,°C	W, %	Emission	T,°C	W, %
Steppe	23.2 ± 3.4 *a*	11 ± 1 *b*	27 ± 1 *b*	17.6 ± 6.7 *a*	17 ± 1 *b*	13 ± 4 *ab*	20.0 ± 7.5 *a*	18 ± 0 *c*	19 ± 5 *ba*
Forest	12.1 ± 9.3 *bc*	9 ± 0 *c*	36 ± 2 *a*	9.6 ± 5.2 *ab*	19 ± 2 *b*	21 ± 3 *a*	16.7 ± 2.4 *a*	19 ± 0 *c*	30 ± 1 *a*
Fallow	3.3 ± 1.1 *c*	15 ± 2 *a*	20 ± 4 *b*	2.0 ± 0.8 *b*	24 ± 2 *a*	12 ± 4 *b*	5.6 ± 2.0 *b*	33 ± 1 *a*	18 ± 2 *b*
Urban	15.7 ± 2.6 *ab*	13 ± 2 *ab*	27 ± 7 *b*	9.6 ± 3.0 *ab*	20 ± 4 *b*	13 ± 7 *ab*	18.7 ± 2.1 *a*	22 ± 3 *b*	24 ± 7 *ab*

Table 3. Temporal changes of soil microbial biomass carbon (C_{mic}, μg C g^{-1} soil), basal respiration (BR, μg CO_2-C g^{-1} soil h^{-1}) and specific respiration of microbial biomass (qCO_2, μg CO_2-C mg^{-1} C_{mic} h^{-1}) of Chernozems (0-10 cm) in different ecosystems (Kursk region, 2015). The values with different letters were significantly (p ≤0.05) differ for each parameter separately

Ecosystem	May			June			July		
(n = 5)	C_{mic}	BR	qCO_2	C_{mic}	BR	qCO_2	C_{mic}	BR	qCO_2
Steppe	1710 ± 370 *a*	1.01 ± 0.17 *a*	0.60 ± 0.10 *b*	1132 ± 167 *a*	1.22 ± 0.39 *a*	1.07 ± 0.23 *ab*	1414 ± 233 *a*	1.64 ± 0.6 *a*	1.14 ± 0.32 *a*
Forest	1660 ± 488 *a*	0.92 ± 0.11 *a*	0.59 ± 0.18 *b*	1369 ± 342 *a*	0.69 ± 0.04 *b*	0.52 ± 0.10 *c*	1356 ± 378 *a*	1.16 ± 0.12 *a*	0.91 ± 0.26 *a*
Fallow	372 ± 130 *b*	0.28 ± 0.10 *b*	0.76 ± 0.21 *b*	310 ± 45 *b*	0.29 ± 0.03 *b*	0.92 ± 0.11 *b*	397 ± 33 *b*	0.41 ± 0.09 *b*	1.05 ± 0.26 *a*
Urban	284 ± 101 *b*	0.48 ± 0.15 *b*	1.72 ± 0.30 *a*	439 ± 158 *b*	0.55 ± 0.19 *b*	1.28 ± 0.26 *a*	351 ± 140 *b*	0.43 ± 0.07 *b*	1.36 ± 0.50 *a*

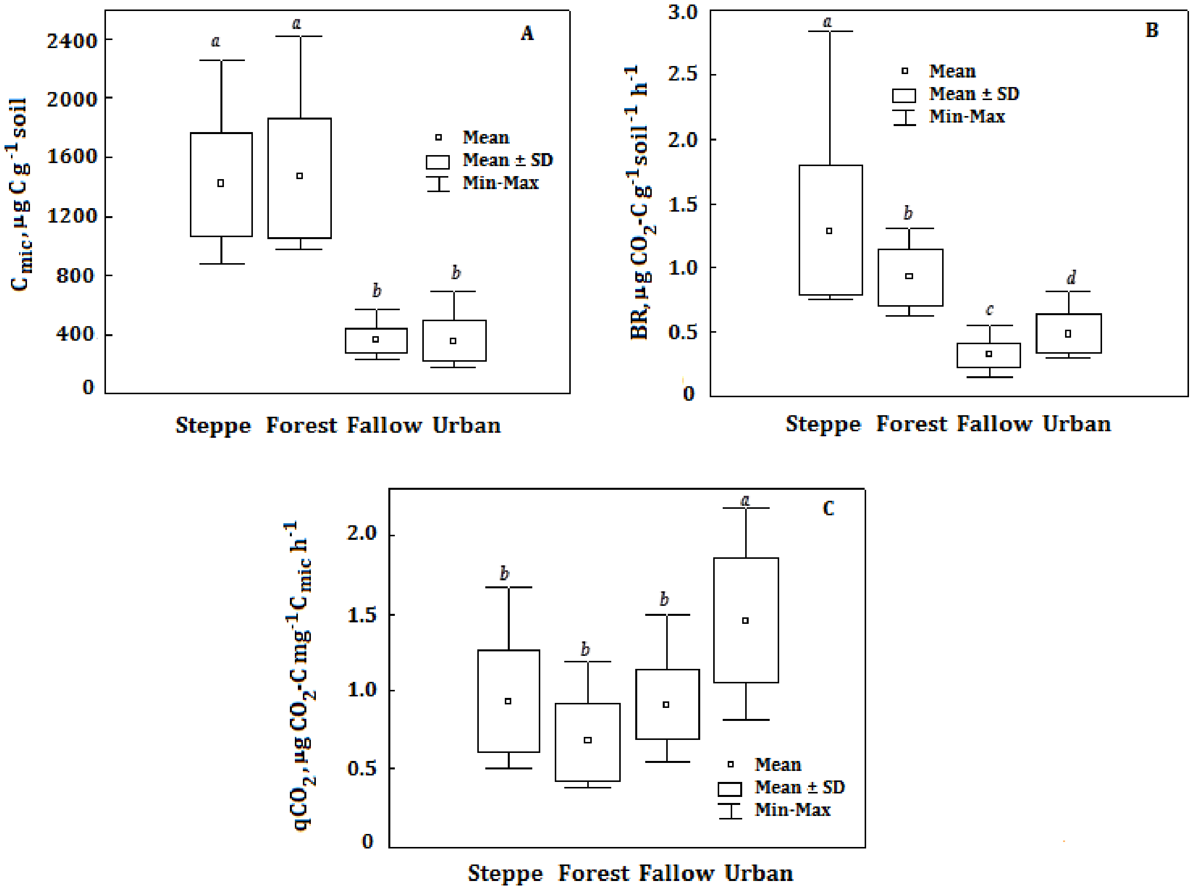


Figure 3. Distribution (box-plot) of soil microbial biomass carbon content (C_{mic}, µg C g^{-1} soil), basal respiration (BR, µg CO_2-C g^{-1} soil h^{-1}) and specific respiration of microbial biomass (qCO_2, µg CO_2-C mg^{-1} C_{mic} h^{-1}) in Chernozems (0-10 cm) of different ecosystems (n = 15, May-June-July, 2015). The values with different letters were significantly ($p \leq 0.05$) different each other

Table 4. Soil organic carbon content (C_{org}), soil microbial biomass carbon (C_{mic}), C_{mic} portion in C_{org}, the highest inhibition of substrate-induced respiration (SIR) by streptomycin and cycloheximide both, fungi / bacteria (F / B) and C_{fungi} / C_{org} ratios in different ecosystems of typical Chernozems

Ecosystem	C_{org}, %	pH_w	C_{mic}, µg C g^{-1} soil	C_{mic} / C_{org}, %	F / B	SIR inhibition, %	C_{fungi} / C_{org}, %
Steppe	5.57	6.24	1606 ± 130	2.9	3.8 ± 1.2	51	2.4 ± 0.43
Urban	1.71	7.97	191 ± 32	1.1	3.4 ± 0.1	41	1.0 ± 0.0

The soil MR of steppe, forest and urban ecosystems for three months of observation was varied from 4.8 (urban) to 17.5 (forest) g CO_2 m^{-2} d^{-1} and amounted in average 6.9, 9.1 and 10.8 g CO_2 m^{-2} d^{-1} for urban, steppe and forest, respectively, however these values were not significantly differ (data not shown). The MR portion in total soil CO_2 emission in May was varied from 27% in urban to 91% in forest (Figure 4). The highest portion of MR was found in forest, it was 76 и 91% for two replicates (points). The highest difference of MR between replicates was in forest and urban. In the first replicate of forest it was a rich undergrowth of shrubs (more roots), and in the second replicate it was rare (less roots). The first replicate of urban industrial zone was covered by rich grasses and had sod cover (less MR), the second replicate was almost without grass cover (more MR). The MR portion in total soil CO_2 emission for the three studied months was the highest in forest and amounted in average 83% (Figure 5). The MR portion in total soil CO_2 emission of steppe and urban was less and amounted in average 51 and 60%, respectively.

Between MR and soil total CO_2 emission (or BR, C_{mic} and soil water content) the positive correlation was found (r = 0.48, 0.51, 0.34, respectively) for studied months. Between MR and soil temperature the correlation was weak (r = 0.06). Between MR (*in situ*) and BR (*lab test*) was revealed the regression relationship with high satisfactory R^2 (Figure 6). So, the relationship might be allowed to predict soil MR (time- and labor-consuming procedure) by soil BR measurement.

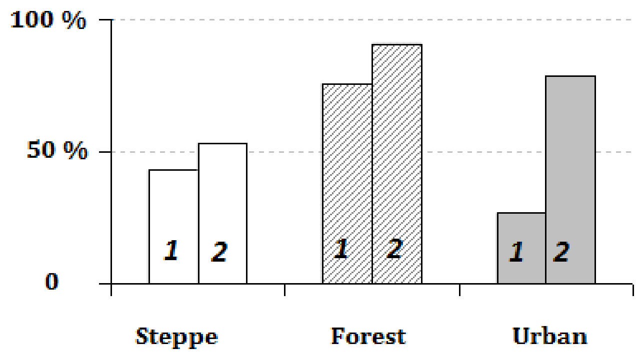


Figure 4. The portion of soil microbial respiration in total soil CO_2 emission of Chernozems in different ecosystems (1 and 2 are numbers of measurement point, May, 2015)

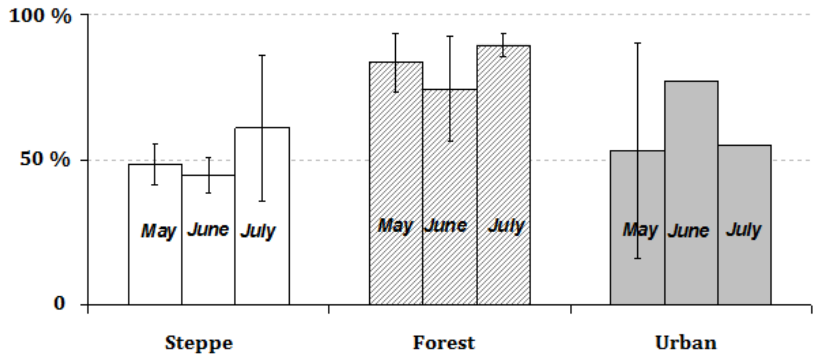


Figure 5. The portion of soil microbial respiration in total soil CO_2 emission of Chernozems in different ecosystems (n = 2, May-June-July, 2015)

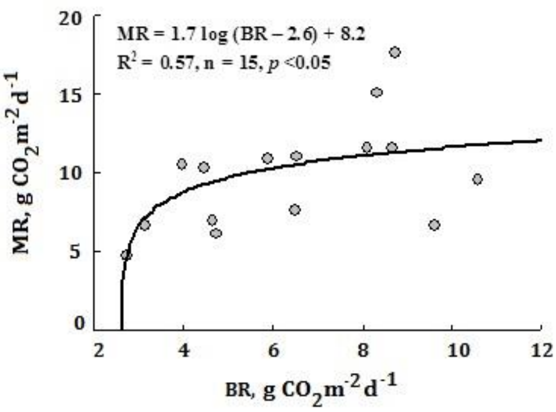
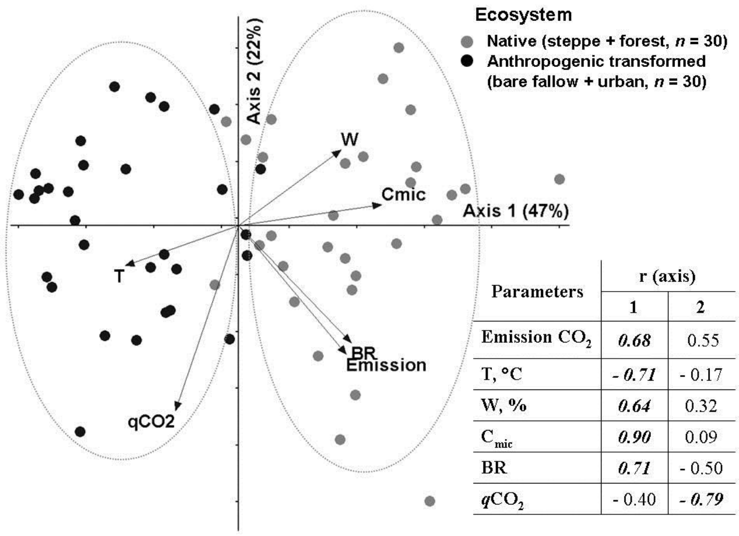


Figure 6. Relationship between microbial (MR) and basal (BR) respirations in Chernozems of steppe, forest and urban ecosystems

A principal component analysis showed that the first and second axes (components) produce 47 and 22% of the experimental data variation, respectively (Figure 7). The highest correlation of the first axis was found with the content of C_{mic}, BR, CO_2 emission, soil temperature and moisture. The highest correlation of the second axis was found with qCO_2 value. The first axis can be considered as the gradient of ecosystem changes. The soils of undisturbed (natural) ecosystems are mainly collected in the right part of figure, and the soils of disturbed (anthropogenically transformed) ecosystems are located in the left part. This might be indicated a more "optimum" functioning of the soil microbial community in Chernozems of natural ecosystems.



Parameters	r (axis)	
	1	2
Emission CO_2	***0.68***	0.55
T, °C	***- 0.71***	- 0.17
W, %	***0.64***	0.32
C_{mic}	***0.90***	0.09
BR	***0.71***	- 0.50
qCO_2	- 0.40	***- 0.79***

Figure 7. PCA ordination of Chernozems CO_2 emission (g CO_2 m^{-2} d^{-1}), hydrothermal (T, W) and microbiological (C_{mic}, µg C g^{-1} soil; BR, µg CO_2-C g^{-1} soil h^{-1}; qCO_2, µg CO_2-C mg^{-1} C_{mic} h^{-1}) parameters of natural and anthropogenically transformed ecosystems (Kursk region)

Conclusion

Along a gradient of Chernozems ecosystems (steppe, forest, fallow, urban) the significant decrease of soil C_{mic}, BR and C_{fungi} / C_{org} ratio was found (by 2-4 times less), while the qCO_2 value increased. It might be illustrated an "deterioration" of soil microbial community functioning under anthropogenic transformation of terrestrial ecosystems. Soil basal respiration (*lab test*) might be characterized soil microbial respiration *in situ*.

Acknowledgements

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Benzo[a]pyrene contamination in Rostov Region of Russian Federation: A 10-year retrospective of soil monitoring under the effect of long-term technogenic pollution

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Abstract

The aim of the current work was to study the main tendencies in the accumulation and distribution of benzo[a]pyrene in soils of the affected zone of the Novocherkassk regional power plant. Studies were conducted on the soils of monitoring plots subjected to Novocherkassk regional power plant emissions. Monitoring plots were established at different distances from the Novocherkassk regional power plant (1.0–20.0 km). Regularities in the accumulation and distribution of benzo[a]pyrene in chernozemic, meadow-chernozemic, and alluvial soils under the effect of aerotechnogenic emissions from the Novocherkassk regional power plant have been revealed on the basis of long-term monitoring studies (from 2002 to 2011). The tendencies in the distribution and accumulation of BaP in the studied soils coincided during the 10 years of monitoring studies. It has been found the 5-km zone to the northwest from the power station, which coincides with the predominant wind direction, is most subjected to contamination by benzo[a]pyrene, with the maximum accumulation at a distance of about 1.6 km from the source. Dynamics of pollutant accumulation in soils depends on number of Novocherkassk regional power plant emissions. The content of benzo[a]pyrene in the soil is an indicator of the technogenic load impact on the areas, for which the combustion products of hydrocarbon fuel are the major pollutants. A gradual decrease of the pollutant content in the soils was revealed during the period from 2002 to 2011. It explained by the significant decrease in the volume of pollutant emissions from the plant and the self-purification capacity of soils and mechanisms of benzo[a]pyrene degradation.

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Introduction

The assessment of the environmental status of soils as a central link of ecosystems is an essential parameter in the system of environmental monitoring. The soil is the central ecosystem component depositing pollutants. The regular observation of the accumulation and distribution of anthropogenic pollutants in the

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soil is an essential problem of soil science. The improvement of the state of the environment under pollution is possible only after long-term monitoring studies for revealing the character and nature of pollution, the composition of pollutants, their diversity, and mechanisms for the accumulation and transformation of pollutants in the studied biogeocenosis (Cristale et al., 2012; Tobiszewski and Namiesnik, 2012). The most optimal methods for the restoration of the area subjected to technogenic contamination can be found only on the basis of large-scale monitoring studies and the investigation of the main tendencies in the accumulation of pollutants (Antizar-Ladislao et al., 2006; Augusto et al., 2013; Minkina et al., 2012; Oros et al., 2013).

Polycyclic aromatic hydrocarbons (PAHs) are among the most hazardous and widely distributed soil pollutants characterized by increased toxicity and carcinogenicity. The content of PAHs in all natural objects is subject to mandatory control throughout the world, which is regulated by legislations of different countries (GOST 17.4.1.02.-83, 2004; GOST 17.4.3.06-86, 1986; Jian et al., 2004; Wenzl et al., 2006).

Benzo[a]pyrene (BaP) is most frequently considered as the main marker of soil contamination by PAHs, because this is the most prevalent PAH characterized by a very high persistence in environmental objects and elevated carcinogenicity and mutagenicity (Jian et al.,, 2004). BaP is a compound of hazard class 1; it is included in the group of superecotoxicants, and its content in all objects of the ecosystem is subject of mandatory control (Tobiszewski and Namiesnik, 2012; Wenzl et al., 2006). In Russia, the maximum permissible concentration (MPC) of BaP is 0.02 mg/kg for all soils; in other countries, this value varies in the range of 0.1–2.7 mg/kg.

The monitoring studies of environmental pollution with PAHs has been performed in many countries over tens of years. A number of works well with the study of the state of the areas subjected to technogenic contamination with PAHs (Callén et al., 2013; Pereira et al., 2013; Singh et al., 2013; Sushkova et al., 2015; Sushkova et al., 2015; Witter et al., 2014; Li et al., 2006; Yam and Leung, 2013; Zhu et al., 2014). The contamination is of technogenic origin in all the cases.

Active sources of environmental pollution with PAHs include enterprises of energy industries, especially great thermal stations (DEFRA and EA 2002; Sushkova et al., 2015; Witter et al., 2014; Yam and Leung, 2013). The Novocherkassk Regional Power Plant (NRPP) is one of the greatest thermal power stations not only in Russia, but also in Europe. This is an enterprise of hazard class 1, which was set in operation in 1965–1971. At present, it includes eight working blocks and is the main source of electrical energy in Rostov oblast. Coal and natural gas are the major fuel types for the station. The height of the first chimneystack is 185 m; the three other stacks are 250 m high.

Ecological monitoring performed since 2000 showed that the NRPP is the main pollution source of the atmospheric air not only in the city of Novocherkassk, but also in the entire Rostov oblast, and makes the major contribution to the environmental pollution in this region. The aim of the current work was to study the main tendencies in the accumulation and distribution of BaP in soils of the affected zone of the NRPP.

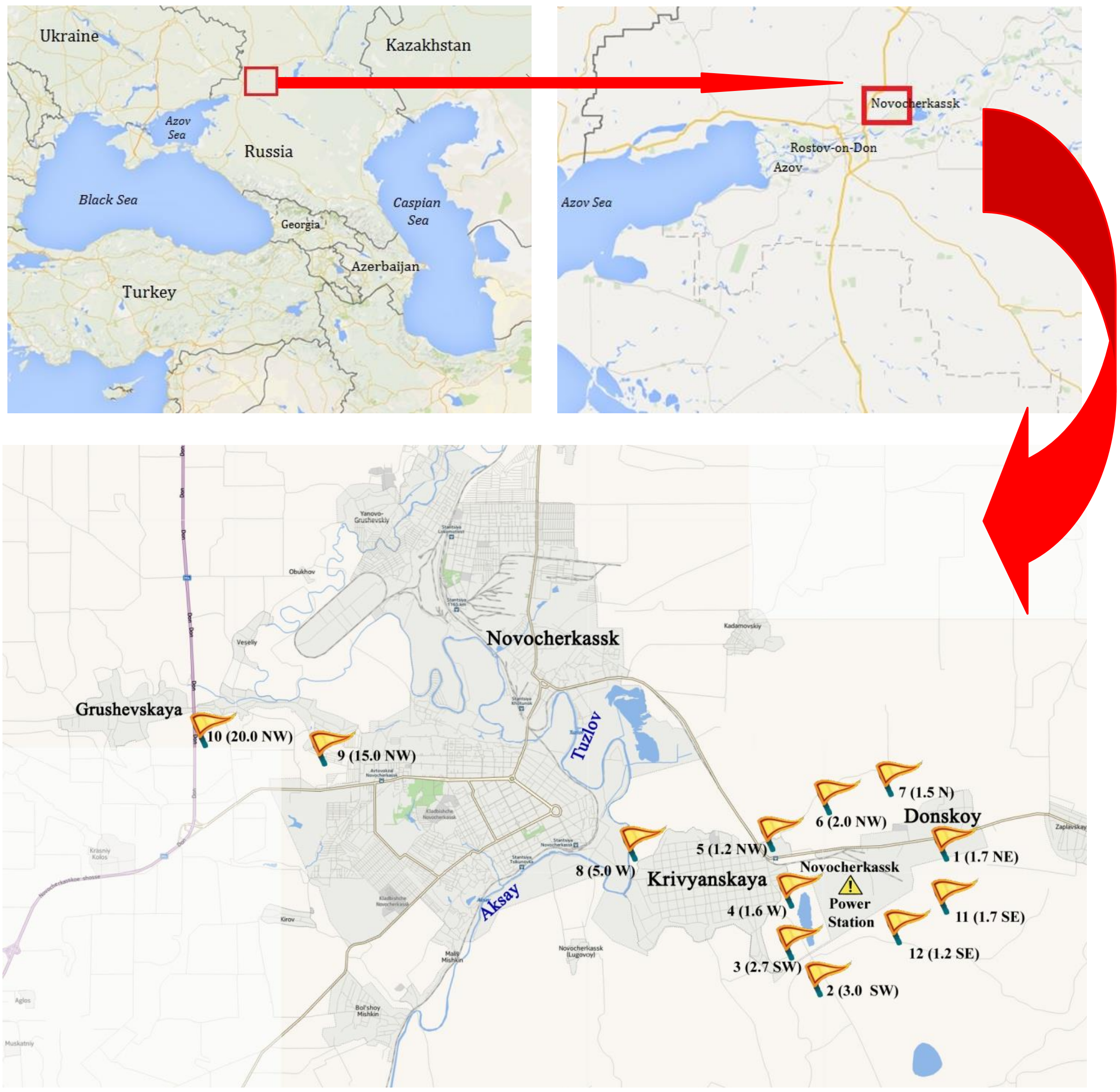
Material and Methods

The main objects of study were soils in the affected zone of the NRPP. The satellite images of the NRPP and its affected zone, as well as the locations of monitoring plots, are given in Figure 1. They coincided with the air sampling sites for the ecological certificate of the plant (plots 1, 2, 3, 5, 6, 7) (Figure 1). The most attention was paid to the main wind direction from the contamination source to the northwest through the residential areas of Novocherkassk (plots 4, 8, 9, 10) (Table 1). The monitoring plots were located on virgin lands or fallow areas. The soil cover in the region under study consisted of ordinary chernozems, meadow-chernozemic soils, and alluvial meadow soils.

Table 1. Numbers of monitoring plots and their code including distance (km) and the direction from the Novocherkassk Regional Power Plant (NRPP), and also soil type on a plot

Plot No.	Code	Soil type	Plot No.	Code	Soil type
Group I			Group II		
5	1.2 NW	Co	1	1.0 NE	Co
4	1.6 NW	Co	7	1.5 N	Co
8	5.0 NW	MCS	6	2.0 N	MCS
9	15.0 NW	Co	3	2.7 SW	MCS
10	20.0 NW	Co	2	3.0 SW	AS

Co: Chernozem ordinary; MSC: Meadow chernozem soil; AS: Alluvial soil



Plot No.	The direction and distance from NRPP	Plot No.	The direction and distance from NRPP
1	1.0 km on the northeast (NE)	6	2.0 km on the northwest (NW)
2	3.0 km on the southwest (SW)	7	1.5 km to the north (N)
3	2.7 km on the southwest (SW)	8	5.0 km on the northwest (NW)
4	1.6 km on the northwest (NW)	9	15.0 km on the northwest (NW)
5	1.2 km on the northwest (NW)	10	20.0 km on the northwest (NW)

Figure 1. Schematic map of monitoring plots in the zone affected by the Novocherkassk Regional Power Plant (NRPP)

Most of the area in the affected zone of the NRPP is occupied by calcareous ordinary chernozem (Co); meadow-chernozemic soil (MCS) (plot 3SW) and alluvial soil (AS) also occur in the Tuzlov River floodplain of the studied zone (Table 2).

The Co and MCS have thick humus horizons (70−100 cm), relatively high content of humus (4.1−5.0%) and high cation exchange capacity (CEC) (31.2−47.6 cmol(+)/kg), including a high content of exchangeable calcium (76−90% of total exchangeable cations), and neutral or weakly alkaline reaction (pH_{water} 7.4−7.7) (GOST 26423-85, 1985). According to particle size distribution, they belong to heavy loamy and light clayey varieties developed on calcareous loess-like rocks. The climatic index of biological productivity (Bc) is 90−100 under natural conditions and 170−175 under optimum wetting conditions. The sufficient amount of heat and precipitation forms soils with high natural fertility, and the enrichment with carbonates from the parent rocks favors the development of high buffering properties. The alluvial soil has light texture, thinner humus horizon (40−60 cm), lower humus content (lower than 3.1%) and lower CEC (10.9 cmol(+)/kg) with a relatively high content of exchangeable calcium.

Table 2.Properties of the Novocherkassk Regional Power Plant (NRPP) emissions zone soils (an average for 2002-2011)

Number of montoring plot	Soil	Physical clay, %	Clay, %	C_{org}, %	pH	$CaCO_3$, %	CEC, cmol(+) kg^{-1}
1	Low-humus medium-thick calcareous clay loamy ordinary chernozem on loess-like loams	52.0	27.0	4.3	7.6	0.5	35.0
2	Low-humus calcareous sandy alluvial meadow soil on alluvial deposits	7.0	3.0	3.1	7.5	0.4	10.9
3	Low-humus silty clayey flood-plain meadow chernozemic soil on alluvial deposits	67.0	37.0	4.6	7.3	0.2	44.8
4	Low-humus medium-thick calcareous clay loamy ordinary chernozem on loess-like loams	55.0	29.0	4.6	7.5	0.7	31.2
5	Low-humus medium-thick calcareous clay loamy ordinary chernozem on loess-like loams	53.0	27.0	4.3	7.5	1.0	35.7
6	Low-humus medium-thick clay loamy meadow chernozemic soil on loess-like loams	55.0	30.0	4.1	7.7	0.8	32.4
7	Low-humus medium-thick calcareous clay loamy ordinary chernozem on loess-like loams	51.0	27.0	4.1	7.6	0.7	31.3
8	Low-humus medium-thick clay loamy meadow chernozemic soil on loess-like loams	60.0	32.0	5.0	7.4	0.4	47.6
9	Low-humus medium-thick calcareous clay loamy ordinary chernozem on loess-like loams	52.0	30.0	4.2	7.6	0.6	31.4
10	Low-humus medium-thick calcareous clay loamy ordinary chernozem on loess-like loams	53.0	28.0	4.6	7.6	0.5	36.0

Soil samples were taken daily in June during 10 years from 2002 to 2011. The samples were taken by layers, from depths of 0 to 5 and 5 to 20 cm. Soil samples were selected and prepared for the chemical analysis according to GOST 17.4.4.02-84 (GOST 17.4.1.02.-83, 2004) requirements. The samples were used for the determination of soil texture by the Kachinskii method (Gabov and Beznosikov, 2014; Directive document 52.10.556-95, 2002).

Soil samples were taken annually and prepared for chemical analysis in accordance with the current requirements (Pikovskii, 1993; Sokolov, 1966; ISO, 2005). BaP was extracted from the soils of the objects under study by the standard method using for the removal of the interfering soil components by saponification (Directive document 52.10.556-95, 2002).

A 1-g of the prepared soil was put into a pear-shaped flask for rotary evaporator; 20 mL of 2% KOH solution in ethanol was added, and the mixture was refluxed on a water bath for 3 h. The saponificaiton of lipids and gummy soil components occurred during the refluxing, which increased the recovery of PAHs and reduced the amount of coextracted substances in the extract. The supernatant was decanted into an Erlenmeyer flask, and 15 mL of n-hexane and 5 mL of distilled water were added for the better separation of the layers. The mixture was shaken on a rotary shaker for 10 min and transferred into a dividing funnel. The hexane layer was poured into a separate vessel. The residue in the flask was extracted twice more in a similar way. The combined hexane extract was washed with distilled water to neutral pH (using litmus as an indicator), transferred into a dark vessel with a close lid, and desiccated by adding 5 g of anhydrous Na_2SO_4. After exposure at +5°C for 8 h, the desiccated extract was decanted into a dry round-bottomed flask and evaporated to dry on a rotary evaporator at a bath temperature of 40°C. The dry residue was dissolved in 1 mL of acetonitrile.

The content of BaP in the test samples was determined by the external standard method (Anonymous, 2008). The content of BaP in the soil was calculated from the equation

$$C_s = k\, S_i \times S_{st} \times V/(C_{st} \times m) \qquad (1)$$

where Cs is the content of BaP in the soil sample (μg/kg); Sst and Si are the BaP peak areas for the standard solution and the sample, respectively; Cst is the concentration of the standard BaP solution (μg/kg); k is the recovery factor of BaP from the sample; V is the volume of the acetonitrile extract (mL); and m is the mass of the sample (g).

From the results of determining BaP concentrations in the upper and lower soil layers (C_{0-5} and C_{5-20}, μg/kg, respectively), the weighted average concentrations of BaP in the 0- to 20-cm layer (C_{0-20}, μg/kg) were calculated from the equation

$$C_{0-20}=(5C_{0-5}+15C_{5-20})/20 \quad (2)$$

The vertical distribution coefficients of BaP between the upper and lower layers (Kd) were calculated from the equation

$$K_p=15*C_{5-20}/5*C_{0-5} \quad (3)$$

The averaged BaP distribution coefficients between the soil layers were also determined for each monitoring plot during the period from 2008 to 2010 (the period of the maximum stabilization of fallouts), and a correlation between these values and different physicochemical and agrochemical parameters of soils was established. The obtained results were processed by mathematical statistics methods using Microsoft EXEL and SigmaPlot 2011 software.

Results

Dynamics of solid emission products and the composition of pollutants

In the later 1990s, the total volume of emissions from the NRPP reached 139 thousand tons. Due to the re-equipment of the NRPP, which started in 2000, the proportion of gas in the fuel exceeded 40% in 2004. This resulted in a reduction of solid emissions into the atmosphere down to 54 thousand tons, i.e., more than twice (Figure 2). In the following two years, the annual volume of solid emissions increased and remained within the range of 83–101 thousand tons during the next five years (up to 2011) (Ecological messenger of Don..., 2012).

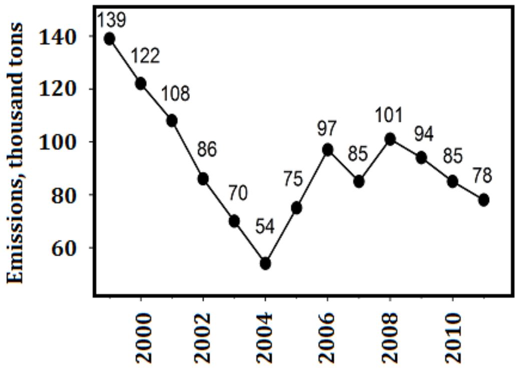


Figure 2. Dynamics of average pollutant emissions value from the Novocherkassk Regional Power Plant (NRPP) between 1999 and 2011 (according to Ecological Messenger of Don, 2012)

The emissions from the NRPP mainly consist of processed coal ash. The coals furnished to the NRPP are enriched with a wide range of organic and inorganic toxic substances (Mandzhieva et al., 2014). The main components of the NRPP emissions are ash, sulfur monoxide, nitrogen oxides, soot (more than 30 t/year), vanadium pentoxide (about 8 t/year), iron oxide (more than 5 t/year), chromic anhydride (about 0.1 t/year), hydrogen fluoride (7 kg/year), etc. Ash retains up to 85% of the chemical elements that were present in the original coal (Gorobtsova et al., 2005; GOST 14.4.3.06-86, 1986; Hybholt et al., 2011; Gennadiev et al., 2004). It was calculated that PAHs make up about 10% of the total annual emission from the NRPP (about 90 thousand tons) (Antizar-Ladislao et al., 2006). Some authors indicate that ash can contain up to 60% PAHs (Gorobtsova et al., 2005), and BaP composes up to 10% of them (Gennadiev et al., 1989).

Dynamics of the content and distribution of BaP in soils of the affected zone of the NRPP

It was found that the concentration of BaP in soils of the affected zone of the NRPP varied in a wide range: from 11 to 423 μg/kg in the 0- to 5-cm layer and from 5 to 249 μg/kg in the 5 to 20-cm layer (i.e., in the ranges of 0.6–21 and 0.3–11 MPC, respectively). On most of the plots, the concentrations of BaP in the both soil layers varied synchronously.

The weighted average BaP concentrations in the 0- to 20-cm layer of soils on the monitoring plots during the period from 2002 to 2011 are shown in Figure 3. The results for the soils located along the predominant wind direction (to the northwest) and within a radius of 1–3 km from the plant in the northern, northeastern, and southwestern directions are shown separately (Figure 3A–3C).

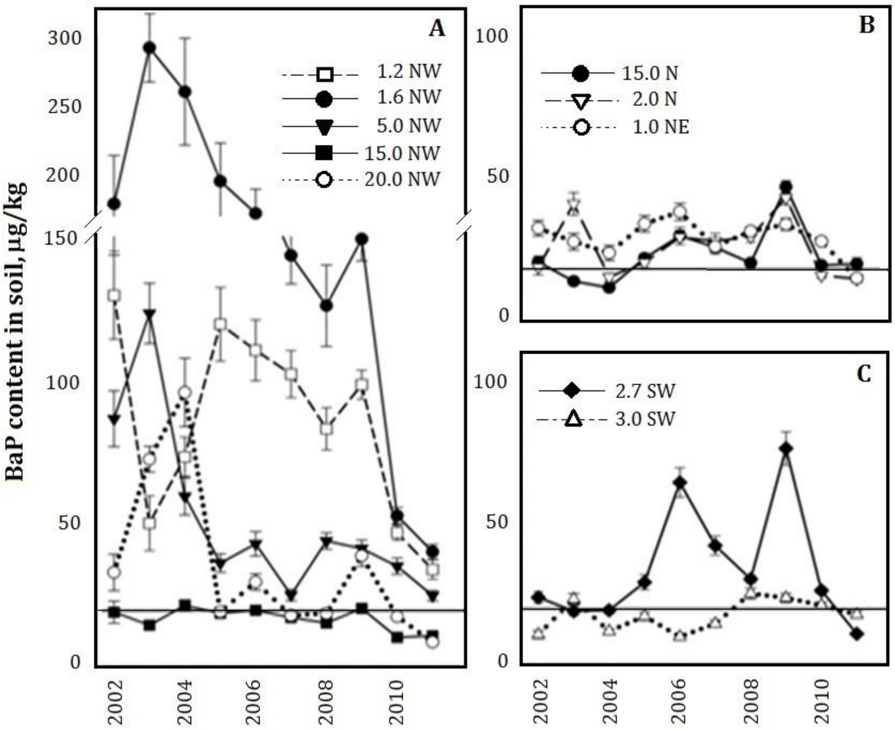


Figure 3. Dynamics of BaP average concentrations in 20-cm layer of soil monitoring plots in the Novocherkassk Regional Power Plant (NRPP) emission zone on the north-west - through the prevailing direction of the wind rose (A), as well as around NRPP on north / northeast (B) and southwest (C) directions. The line indicates the level of MPC in the soil.

The analysis of the results shows that the accumulation of BaP in the soils of the studied area depends on the distance of the controlled plots from the emission source and their location with respect to it. The major part of pollutants accumulates in the soils of the northwestern direction from the NRPP, which coincides with the main direction of winds (Figure 3A). The most significant pollution of soils in this direction is observed within a radius of 5 km, with a maximum at 1.6 km, where $C_{0\text{-}20}$ reached 125–300 µg/kg (6–15 MPC) and $C_{0\text{-}5}$ reached 423 µg/kg (22 MPC) in 2002–2003. When the distance from the emission source increases, the level of contamination with BaP gradually decreases to a minimum at 15 km from the plant, where $C_{0\text{-}20}$ varies in the range of 1–2 MPC and $C_{0\text{-}5}$ does not exceed 2.6 MPC.

In all the soils located along the predominant wind direction, an active decrease of BaP content in the entire 0- to 20-cm layer was observed during the 10-year-long period of observations. This tendency is most manifested in the soil of plot 4 with the maximum level of contamination (Figure 3A), where the concentration of BaP decreased by more than 7 times: from 290 to 40 µg/kg. An almost similar decrease of $C_{0\text{-}20}$ (from 125–130 to 25–25 µg/kg, i.e., by 4–5 times) was observed on plots 1,2nw and 5nw.

In the most remote point of group I (20NW), as well as in almost all soils of group II (Figure 3B, 3C), the content of BaP remained low (at 0.5–2 MPC); only on plot 2,7nw, it reached 3–4 MPC in some years. In points 20NW and 1ne, a tendency of slow decrease in the concentration of BaP (by about 2 times for 10 years) was observed; on the other plots, the most significant decrease in BaP concentration was observed only in the last years (from 2009 to 2011).

The revealed tendencies in the decrease of BaP concentration in the soils of monitoring plots are analogous to those observed for the decrease in the volume of solid emissions from the plant during the period from 1999 to 2004. In most of the curves, the peaks of increasing and decreasing BaP concentration coincide with each others and with the peaks of soot emission but are delayed from the emission dynamics by about a year. After 2004, the decrease of BaP concentration in the soil continued, in spite of the relative stabilization of emissions from the NRPP, and accelerated during the period of the last decrease in the volume of solid emissions beginning from 2008.

From the data shown Figure 3, the period of half-decrease of BaP concentration in the soils (T_{50}) could be estimated. In points 1.2NW, 1.6NW, 5.0NW, and 15.0NW, where the weighted average concentration of BaP in the 0- to 20-cm layer increased to 100–300 µg/kg, the value of T_{50} varies in the range of 1–5 years. In the two points with low contamination levels (20NW and 1.0NE), where the maximum BaP concentration did not exceed 20–40 μg/kg, this value was about 10 years. In the other soils subjected to slight contamination, the rate of decrease in BaP concentration could not be determined.

The concentrations of BaP in the 0- to 5- and 5- to 20-cm layers of soils on the monitoring plots averaged for the period of 2005–2011, when the annual emissions from the power plant were stabilized at a level of 75–101 thousand tons. In all the sampling points on chernozemic and meadow-chernozemic soils, the averaged concentrations of BaP in the upper soil layer exceed its contents in the lower layer by 1.5–2 times. An exception is point 2 on sandy alluvial soil located in the Tuzlov River floodplain, where the concentrations of BaP in the both layers are similar and do not exceed the MPC.

Discussion

It was established that the vertical distribution coefficients of BaP between the 5- to 20- and 0- to 5-cm layers (with account for their thicknesses) in different sampling points vary during the observation period from 0.7 to 5.2 and significantly depend on the soil properties. The highest values of Kd are typical for the sandy soil, and the lowest values are typical for the light clayey soil. The former vary among the years in the range of 1.6–5.2, and the latter vary in the range of 0.8–2.1; in the most common group of loamy soils, Kd varies in the range of 0.7–2.9.

The closest correlations are found between the average coefficients of vertical distribution and the properties of soil during the period of 2008–2010, when the volume of solid emissions was stabilized on the level of 78–101 thousand tons (Figure 4). For this period, close inverse correlations between the Kd values and the contents of physical clay and humus and CEC with the correlation coefficients (R) of 0.93, 0.91, 0.74, and 0.80, respectively (for this data set, the critical R value is 0.632 at p = 0.05) were observed. Analogous, although looser, correlations are traced between these values during other observation periods. For the other properties, correlations with Kd are loose or absent.

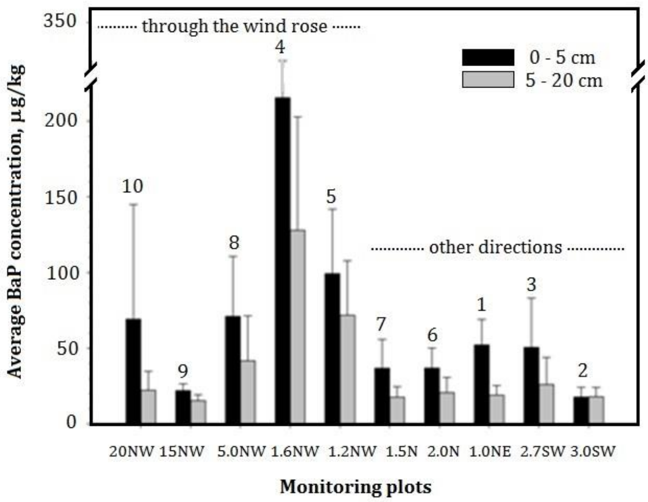


Figure 4. Average BaP concentration in 0-5 and 5-20 cm soil layers of monitoring plots in the Novocherkassk Regional Power Plant (NRPP) emission zone during 2005-2011: in Group I - through the prevailing direction of the wind rose (plots 20NW, 15NW, 5NW, 1.6NW and 1.2NW) and in Group II – around NRPP (plots 1.5N, 2.0N, 1.0NE, 2.7SW and 3.0SW).

Earlier studies showed that the accumulation of BaP in the studied steppe biocenosis is due to the deposition of solid emission products from the NRPP in adjacent areas and depends on the predominant wind direction, which confirms the earlier conclusions (Sushkova et al., 2012, 2015; Gorobtsova et al., 2005; Minkina et al., 2011; Nazarenko et al., 2007). In the current work, the migration and transformation of BaP are considered in more detail.

Our studies showed that, beginning from 2002–2003, the reduction of environmental load is accompanied by a simultaneous decrease in the content of BaP in the both layers of soils on the monitoring plots in the affected zone of the NRPP, especially along the predominant wind direction, where its weighted average concentration reached high values of 100–300 μg/kg. The content of BaP in the both layers remained above the MPC during the entire period of observations. However, in 2011, the concentration of BaP in the soils of most plots decreased to the MPC level; only along the predominant wind direction (within the radius of 5 km), it still exceeded this level by 1.2−2 times.

The abrupt decrease in the content of BaP in the soil of monitoring plot 5nw compared to plot 1,6nw (by more than 3.5 times) indicates that the distribution area of the densest plume containing the maximum amount of pollutant is about 5 km to the northwest, and the maximum fallouts are observed at a distance of about 1.6 km (Sushkova et al., 2012). The areas located around the NRPP up to 3 km in the northern/northeastern and southwestern directions are less contaminated. An exception is the soil on plot 2.7SW, which is the closest to group I, where the concentrations of BaP in the 0- to 20-cm layer reached 60−70 µg/kg in 2006 and 2009.

It should be noted that the soils of the plots located at 1−2 km to the north and northeast of the plant are almost not subjected to the impact of polluting emissions. During the entire period of observations, the content of BaP in the 0- to 20-cm layer of soils on these plots located at a short distance from the plant (1−2 km) exceeded the MPC by no more than 2.5 times (Figure 3C).

The soils on the most remote monitoring plot 10 (20NW) occupy a special place in the description of the affected zone of NRPP. The obtained data indicate the presence of additional sources of pollutant emission near the plot: exhausts from motor vehicles on the M-4 Don highway, which passes at 350m from the sampling site. The plot is located within the V-shaped area enclosed by two highways (the Rostov−Moscow road from the northwest and the Rostov−Novocherkassk road from the southeast), which results in the contamination of soils with vehicle exhausts containing BaP. In addition, this plot is apparently subjected to the plumes from the Novocherkassk dumps, as well as combustion products formed at the stove heating of houses in the village of Grushevskaya. Nonetheless, the accumulation of the pollutant in the soil due to the additional emission sources is significantly lower than that caused by the NRPP.

The period of half-decrease of the pollutant concentration in the most contaminated soils varies in the range of 1−5 years; during the period of 2009−2011, the process was accelerated and T_{50} decreased to 0.1−1 years. On the slightly contaminated soils, T_{50} did not exceed 10 years or could not be determined at all.

The almost simultaneous decrease of BaP concentrations in the upper and lower layers of soil, as well as the acceleration of this process to 2011, indicates the leading role of the microbial degradation of the pollutant in the entire soil layer under study. Although the photo-oxidation of BaP on the soil surface also cannot be excluded (Shabad, 1982), its contribution to the decomposition is apparently minimum because of the shielding of molecules sorbed by soil and soot particles.

It is known that BaP belongs to the persistent pollutants, because microorganisms are incapable of using 4−5-ring PAHs as the only source of carbon and energy. Nonetheless, the microbial degradation of BaP occurs in oxidative conditions under the effect of microorganisms utilizing 2−3-ring PAHs, which usually accumulate in the contaminated soils because of the adaptation of soil microflora (Shabad, 1982). The accelerated degradation of BaP, which is observed in the most contaminated soils, is due to the faster adaptation of microorganisms in the presence of the selective factor (Gabov et al., 2007; Galiulin et al., 2002). The slower decrease of BaP concentration in the slightly contaminated soils can be related to their low availability to biodestructors due to the strong sorption by soil humus or pyrogenic particles (Augusto et al., 2013). However, the absence of appreciable BaP accumulation in the soil, in spite of the continuing input of soot fallouts in the affected zone of the NRPP, can also argue for the degradation of the pollutant.

The analysis of the physicochemical and agrochemical properties of soils in the studied areas (Table 2) suggests that, in spite of the intensive long-term technogenic load and the high contents of BaP and heavy metals in soils of the studied areas, the high level of fertility still remains in the affected zone of the NRPP, which sustains the ecological balance and forms the basis for the stability and balanced functioning of the ecosystem (Nazarenko et al., 2007; Sushkova et al., 2014; Page et al., 2006).

The comparison of the concentrations of BaP in the 0- to 5- and 5- to 20-cm layers showed that the surface accumulation of BaP prevails in the chernozemic soils of heavy texture occurring in the affected zone of the NRPP. However, the relatively high vertical distribution coefficients of the pollutant between these layers, which exceed 1 in most cases and reach 2.9, indicate the migration of BaP throughout the soil profile, at least within the 0- to 20-cm layer. The most intense migration of BaP proceeds in the floodplain soil of the Tuzla River floodplain, where the value of Kd reaches 5.2. A close correlation is found between the vertical migration coefficient of BaP and the contents of physical clay, clay, and humus and CEC in soils of the all monitoring plots (Figure 5).

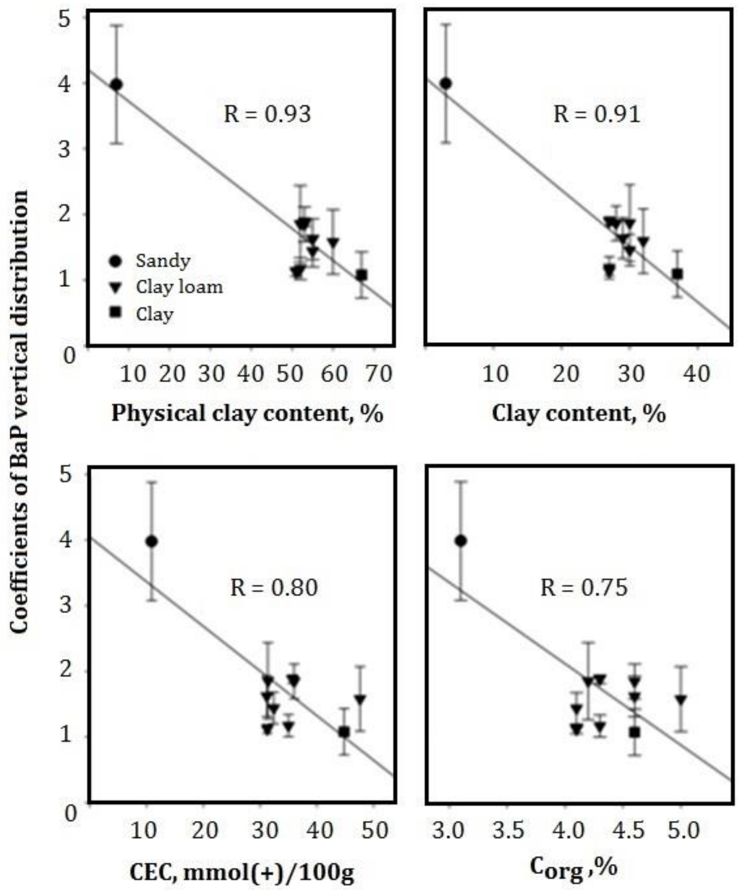


Figure 5. Correlations between the average coefficients of BaP vertical distribution in the 5-20 cm soil layer and 0-5 cm soil layer and the properties of monitoring plots soil

The low mobility of BaP in the zonal chernozemic soils is due to its low solubility in water, high lipophilicity, and increased capacity of being sorbed by soil organic matter, whose content is maximum in the fine fraction of soil and depends on the content of fine particles. In the less humified soil of light texture, the migration of BaP is appreciably enhanced.

The obtained data agree with the results of studies (Gabov et al., 2007; Sushkova et al., 2012, 2015; Gorobtsova et al., 2005; Minkina et al., 2011; Nazarenko et al., 2007), which indicate the effect of soil texture on the migration of BaP in the soils of natural and technogenic landscapes.

Conclusion

The tendencies in the distribution and accumulation of BaP in the studied soils coincided during the 10 years of monitoring studies. The toxic emissions from the NRPP are the main factor of technogenic impact on the soils in the region under study; vehicle exhausts can be sources of additional BaP emission. A gradual decrease of the pollutant content in the soils of the studied areas was revealed during the period from 2002 to 2011, which was related to the significant decrease in the volume of pollutant emissions from the plant and the self-purification capacity of soils due to the microbiological and other mechanisms of BaP degradation. In spite of the conservation measures undertaken at the power plant, the atmospheric emissions from the NRPP have still the predominant effect on the environmental situation in the adjacent areas at present.

The particle size distribution in the soils significantly affects the accumulation and differentiation of BaP in the soil profile. A positive correlation of the vertical distribution coefficients of BaP between the 0- to 5- and 5- to 20-cm layers of soil with the contents of physical clay, clay, and humus and CEC is revealed. The elevated values of Kd in the sandy floodplain soil of the Tuzlov River floodplain indicate the possible migration of the hazardous pollutant to the ground and surface waters of the studied region.

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